A.M.A.

Archives OF PATHOLOGY

LINT GRANULOMA — GEORGE AMBRUHN, M.D., ET AL.

THE PATHOLOGIC EFFECTS OF SMOKING ON THE LARYNX — ROBERT F. RYAN, M.D., ET AL.

INBORN ERRORS OF LIPID METABOLISM — DAVID ADLERSBERG, M.D.

HEMANGIOENDOTHELIOMA OF LIVER FOLLOWING THORIUM DIOXIDE ADMINISTRATION — HENRY TERRY, M.D., ET AL.

THE PATHOLOGY OF THE CONDUCTION SYSTEM IN ACQUIRED HEART DISEASE — MAURICE LEV, M.D., ET AL.

RECURRENT CAROTID BODY TUMOR — VICTOR M. ARBAN, M.D.

MYCOSIS FUNGOIDES FOLLOWED FOR FOURTEEN YEARS — JOSEPH G. ABR, M.D., ET AL.

EXPERIMENTAL PROTEINURIA — ROBERT D. COYE Jr., M.D., ET AL.

PULMONARY MICROLITHIASIS — I. D. KENT, M.D., ET AL.

TOXOPLASMOSIS OF NEWBORN — CARTER M. ALEXANDER, M.D., ET AL.

LYMPHOSARCOMA OF THE FEMALE MAMMARY GLAND — SAUL KAY, M.D.

EXPERIMENTAL RENAL INFARCTION — THOMAS L. SAVAN, M.D., ET AL.

COMPLETE CONTENTS ON FIRST PAGE

NOVEMBER 1955

VOLUME 60 NUMBER 5

From Kent et al., p. 556

AMERICAN MEDICAL ASSOCIATION Publication

distinct

cytologic diagnosis in genital and extragenital cancer

PAPANICOLAOU STAINS

EA 50, 100 cc. and 450 cc. bottles
EA 65, 100 cc. bottles
OG 6, 100 cc. and 450 cc. bottles
Harris Hematoxylin (Ortho modification) 100 cc. and 473 cc. bottles
Ortho® Dual-Purpose Cannula
Aspirator bulb (for use with Cannula)

Complete information on request.

Ortho Pharmaceutical Corporation
Raritan, N. J.

Ortho

TABLE OF CONTENTS

VOLUME 60 NOVEMBER 1955 NUMBER 5

ORIGINAL ARTICLES

PAGE

Lint Granuloma

George Amromin, M.D.; Otto Saphir, M.D., and Irene Goldberger, M.A., Chicago 467

The Pathologic Effects of Smoking on the Larynx

Robert F. Ryan, M.D.; John R. McDonald, M.D., and Kenneth D. Devine, M.D., Rochester, Minn. 472

Inborn Errors of Lipid Metabolism

David Adlersberg, M.D., New York 481

Hemangioendothelioma of Liver Following Thorium Dioxide Administration

Henry Tesluk, M.D., and Warren A. Nordin, M.D., Detroit 493

The Pathology of the Conduction System in Acquired Heart Disease

Maurice Lev, M.D., and Paul N. Unger, M.D., Miami Beach, Fla. 502

Recurrent Carotid Body Tumor

Victor M. Arean, M.D., San Juan, Puerto Rico 530

Mycosis Fungoides Followed for Fourteen Years

Joseph C. Aub, M.D., Boston; S. Burt Wolbach, M.D.; B. J. Kennedy, M.D., Minneapolis, and Orville T. Bailey, M.D., Indianapolis 535

Experimental Proteinuria

Robert D. Coye Jr., M.D., Madison, Wis.; David L. Maude, B.A.; Robert F. Dibble, Rochester, N. Y., and Charles L. Yuile, M.D., Los Angeles 548

Pulmonary Microlithiasis

G. Kent, M.D.; E. S. Gilbert, M.D., and H. H. Meyer, M.D., Chicago 556

Toxoplasmosis of Newborn

Carter M. Alexander, M.D., Covina, Calif., and John W. Callister, M.D., Pasadena, Calif. 563

Lymphosarcoma of the Female Mammary Gland

Saul Kay, M.D., Richmond, Va. 575

Experimental Renal Infarction

Thomas L. Gavan, M.D., and Nathan Kaufman, M.D., Cleveland 580

REGULAR DEPARTMENTS

News and Comment 534, 562, 582

A. M. A.
ARCHIVES OF PATHOLOGY

VOLUME 60 NOVEMBER 1955 NUMBER 5

COPYRIGHT, 1955, BY THE AMERICAN MEDICAL ASSOCIATION

EDITORIAL BOARD

PAUL R. CANNON, Chicago, Chief Editor

GRANVILLE A. BENNETT, Chicago
CHARLES E. DUNLAP, New Orleans
WILEY DAVIS FORBUS, Durham, N. C.
STUART LIPPINCOTT, Seattle
SIDNEY C. MADDEN, Los Angeles
WILLIAM MEISSNER, Boston
WILLIAM B. WARTMAN, Chicago
GEORGE H. WHIPPLE, Rochester, N. Y.

AUSTIN SMITH, Editor, A. M. A. Scientific Publications
GILBERT S. COOPER, Managing Editor, Specialty Journals

AMERICAN MEDICAL ASSOCIATION
Scientific Publications

The Journal of the American Medical Association. Weekly. Annual Subscription Price, $15.00.

Quarterly Cumulative Index Medicus. Issued Twice a Year. Subscription Price, Calendar year, $20.00.

A. M. A. Specialty Journals
Monthly

A. M. A. Archives of Internal Medicine. Paul S. Robert B. Lawson, M.D., Chief Editor, 1000 N.W. 17th St., Miami 36, Fla. Price, $12.00; Canada, $12.50; Foreign, $13.50; individual copy, $1.25.

A. M. A. Archives of Dermatology. Herbert Rattner, M.D., Chief Editor, 104 S. Michigan Ave., Chicago. Price, $12.00; Canada, $12.50; Foreign, $13.50; individual copy, $1.25.

A. M. A. Archives of Ophthalmology. Francis Heed Adler, M.D., Chief Editor, 313 S. 17th St., Philadelphia 5. Price, $12.00; Canada, $12.50; Foreign, $13.50; individual copy, $1.25.

A. M. A. Archives of Surgery. Waltman Walters, M.D., Chief Editor, American Medical Association, 535 N. Dearborn St., Chicago 10. Price, $14.00; Canada, $14.50; Foreign, $15.50; individual copy, $1.25.

A. M. A. American Journal of Diseases of Children. Robert B. Lawson, M.D., Chief Editor, 1000 N.W. 17th St., Miami 36, Fla. Price, $12.00; Canada, $12.50; Foreign, $13.50; individual copy, $1.25.

A. M. A. Archives of Pathology. Paul R. Cannon, M.D., Chief Editor, Department of Pathology, University of Chicago, The School of Medicine, 950 E. 59th St., Chicago 37. Price, $8.00; Canada, $8.50; Foreign, $9.25; individual copy, $1.00.

A. M. A. Archives of Neurology and Psychiatry. Tracy J. Putnam, M.D., Chief Editor, 450 N. Bedford Drive, Beverly Hills, Calif. Price, $12.00; Canada, $12.50; Foreign, $13.50; individual copy, $1.25.

A. M. A. Archives of Otolaryngology. George M. Coates, M.D., Chief Editor, 1721 Pine St., Philadelphia 3. Price, $12.00; Canada, $12.50; Foreign, $13.50; individual copy, $1.25.

A. M. A. Archives of Industrial Health. Prof. Philip Drinker, Chief Editor, Dept. of Industrial Hygiene, Harvard University School of Public Health, 55 Shattuck St., Boston 15. Price, $8.00; Canada, $8.50; Foreign, $9.00; individual copy, $1.00.

Checks, money orders, and drafts should be made payable to the American Medical Association, 535 North Dearborn Street, Chicago 10.

Published Monthly by

AMERICAN MEDICAL ASSOCIATION

535 NORTH DEARBORN STREET • CHICAGO 10, ILLINOIS

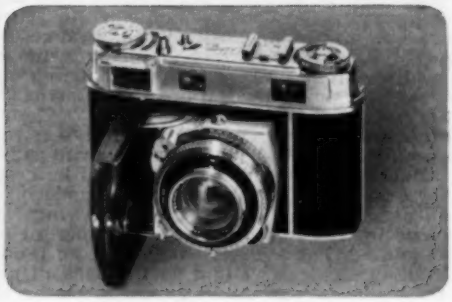
Entered as Second Class Matter Jan. 20, 1926, at the Postoffice at Chicago, Under the Act of March 3, 1879. Annual Subscription, $8.00

A fine new camera ...for medical photographs...

...for advanced amateur picture taking...

The KODAK RETINA IIIc Camera

PRICE $190.



Fine Camera! Equipped with Retina Xenon C Lens, 50mm *f*/2. Interchangeable lens components available.

Built-in photoelectric exposure meter. Coupled rangefinder and viewfinder of new design.

New 10-speed Synchro-Compur Shutter synchronized for regular and electronic flash.

NOTE: Kodak Retina IIc Camera, $139.50—Retina Xenon C Lens, 50mm *f*/2.8, accepts same photo aids as Retina IIIc Camera.

See the new Kodak Retina Cameras at your Kodak photographic dealer's or write for literature.

Fine Photo Aids! Kodak Retina Curtar Lens Component, 35mm *f*/5.6. Kodak Retina Longar Lens Component, 80mm *f*/4.

Kodak Retina Close Range and Viewfinder Kit, Model B, for subject area as small as 3¼ x 5½". Kodak Retina Close-Up Kit, Model B, for subject area as small as 1¼ x 2" (see eye illustration above). Kodak Retina Microscope Adapter Kit, Model B.

Prices include Federal Tax where applicable and are subject to change without notice.

Eastman Kodak Company

***Medical Division,* Rochester 4, N.Y.**

Serving medical progress through Photography and Radiography

Kodak

Instructions to Contributors

Communications regarding editorial management, subscriptions, reprints, etc., should be addressed to Specialty Journals, American Medical Association, 535 North Dearborn Street, Chicago 10.

Articles, book reviews, and other materials for publication should be addressed to the Chief Editor of the Specialty Journal concerned. Articles are accepted for publication on condition that they are contributed solely to that journal.

An original typescript and the first carbon of an article should be provided; it must be double or triple spaced on one side of a standard size page, with at least a 1-inch margin at each edge. An article in English by a foreign author should be accompanied by a draft in the author's mother tongue. Improvised abbreviations should be avoided.

The main title of an article may not contain more than seventy characters and spaces; a subtitle may be of any length. The subtitle should be sufficiently detached from the main title that a punctuation mark as strong as a colon can be used in the Table of Contents to separate it from the main title; it must not begin with "with."

The author's name should be accompanied by the highest academic or medical degree which he holds. If academic connections are given for one author of an article, such connections must be given for all other authors of the article who have such connections.

Typographic considerations necessitate that the first paragraph of an article contain not fewer than thirty words. A Case Report must be preceded by an introductory paragraph containing at least thirty words before the details of the case are given.

Material quoted from another publication must be quoted exactly if the original is in English—spelling, capitalization, punctuation, etc., unchanged. Material taken largely from another publication must be credited to the original author, whether quoted exactly or merely abstracted. If such material is quoted indirectly, the author must be careful to leave no complete sentence of the original unaltered. Use of uncredited quotations will be sufficient cause for rejection of an article.

Each article should be accompanied by a summary in the present tense, presenting the main points of the article, but in somewhat more generalized terms than in the article itself.

If it is necessary to publish a recognizable photograph of a person, the photograph must be accompanied by written permission of the subject himself if an adult, or of the parents or guardian if a child, to publish his photograph. An illustration which has been published in another publication must be accompanied by written permission from the author and the original publisher to reproduce it in an A. M. A. Specialty Journal.

The maximum illustration allowance is ten illustrations within a total of 100 sq. inches or $100, whichever is of greater advantage to the author. When no restrictions are placed by the author upon cropping, reducing, and grouping, the publisher is often able to use twenty or more illustrations in one article with the $100 allowance. Submit sharp prints of the following sizes, 2½″ wide × 2½″ or 3½″ high, 3½″ wide × 2½″ high, and 5½″ or 7″ wide × 3½″ high, to minimize reduction and/or cropping. Oversized originals should be photographed and a print submitted. Large photomicrograph prints will be reduced in scale unless portions to be cropped are indicated by the author.

Any cut-off marks should be made on the margins or mountings rather than on the illustration itself. Charts and drawings should be in black ink on hard, white paper. Lettering must be large enough to permit necessary reduction. Glossy prints of x-rays are requested. Paper clips should not be used on prints, since their mark shows in reproduction, as does writing on the back of prints with hard lead pencil or stiff pen. Labels should be prepared and pasted to the back of each illustration showing its number, the author's name, an abbreviated title of the article, and top plainly indicated. Charts and illustrations must have descriptive legends, grouped on a separate sheet. Tables must have captions. IT IS PREFERRED THAT ILLUSTRATIONS BE UNMOUNTED.

References to the literature should be numbered in the order in which they are referred to in the text or listed in alphabetical order without numbers. A chronological arrangement, with all entries for a given year alphabetized according to the surname of the first author, may be used if preferred. References should be typed on a special page at end of manuscript. They should conform to the style of the Quarterly Cumulative Index Medicus, and must include, in the order given, name of author, title of article (with subtitle), name of periodical, with volume, page, month—day of month if weekly or biweekly—and year. Names of periodicals should be given in full or abbreviated exactly as in the Quarterly Cumulative Index Medicus. Reference to books must contain, in the order given, name of author, title of book, city of publication, name of publisher, and year of publication. Titles of foreign articles, if originally in a generally known Romance or Germanic tongue, must either all be in English translation or all be in the original language. Titles in other languages must be translated. The author must assume responsibility for the accuracy of foreign titles.

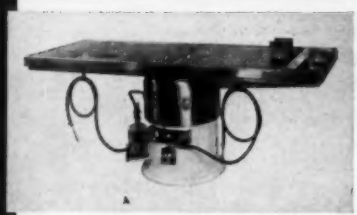
Matter appearing in the A. M. A. Specialty Journals is covered by copyright, but as a rule no objection will be made to its reproduction in a reputable medical journal if proper credit is given. However, the reproduction for commercial purposes of articles appearing in the A. M. A. Specialty Journals, or in any other publications issued by the Association, will not be permitted.

AMERICAN MEDICAL ASSOCIATION

535 North Dearborn Street **Chicago 10**

Designed to meet Pathologists' exacting requirements...

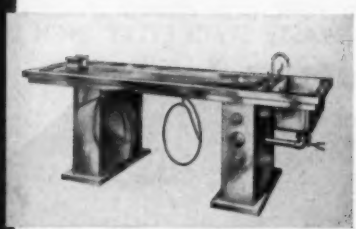
AUTOPSY AND DISSECTING TABLES OF STAINLESS STEEL *by* MARKET FORGE



Model 9M-1485 The Boston

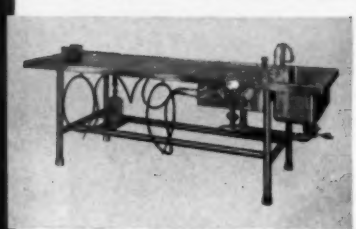
- Stainless steel construction
- Central sanitary base — rotates full 90°
- Complete with flexible rubber hose for irrigation, water-powered aspirator with hose and 2-liter graduated bottle for suction purposes, and rubber headrest
- Removable perforated panels
- Concealed piping

Also Available with Hydraulic Vertical Lift



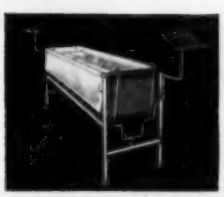
Model 9J-125 The Miami

- All stainless steel construction
- Solid top and integral sink mounted on two pedestals
- Top pitched to foot-end strainered drain
- Sink has knee-operated waste control valve, hot and cold water valves connected to single spout.
- All piping concealed
- Complete with flexible rubber hose for irrigation, water-powered aspirator with hose and 2-liter graduated bottle for suction, and removable hard rubber headrest



Model 9M-1861 The Cambridge

- Combines quality, convenience and minimum cost
- Stainless steel construction
- Solid top with integral sink
- Top is pitched to foot-end strainered sink
- Knee-operated waste control valve, hot and cold water valves connected to single spout
- Complete with flexible rubber hose and nozzle for irrigation, water-powered aspirator with hose and 2-liter bottle for suction, and removable hard rubber headrest



Model 9M-1751 The New York Dissecting Table

- Stainless steel construction
- One of large group of designs regularly manufactured to order.

Market Forge specializes in the manufacture of Dissecting Tables to the custom specifications of schools and hospitals

MARKET FORGE also manufactures a complete line of Mortuary Equipment for Hospitals, Medical Schools and Morgues

quality above all else

since 1897

Complete technical data & Specifications available on request - Send for complete catalog

MARKET FORGE COMPANY EVERETT, MASSACHUSETTS

Paragon Tray Drawer Cabinet

Compact *Low Cost*

U. S. Pat. No. 2,202,047
C101—Tray Drawer Cabinet for 3 x 1 Micro Slides
Capacity 4500—18¾ x 15¾ x 4¾

FOR FILING
MICROSCOPIC SLIDES 3 x 1"
KODACHROME TRANSPARENCIES
2 x 2" SLIDES
LANTERN SLIDES
(up to 3¼ x 4¼)
PETROGRAPHIC SLIDES

When you purchase a
PARAGON TRAY DRAWER CABINET YOU PURCHASE FILING SPACE ONLY NO WASTE SPACE-EVERY INCH USED

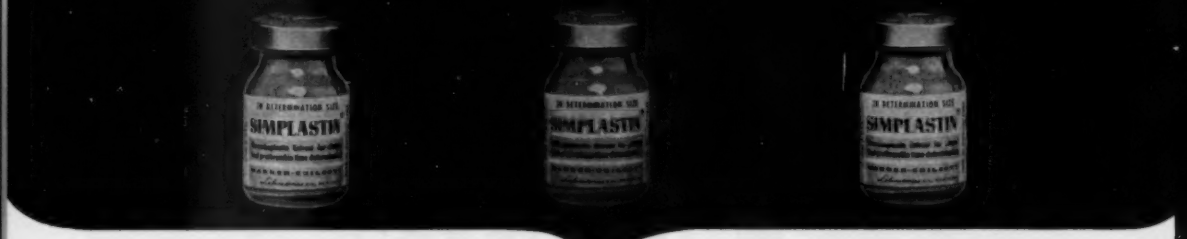
All Paragon Tray Drawer Cabinets are manufactured in standard sizes so that any number of sections may be interlocked to form one cabinet to accommodate any number of varied slides. The dimensions of the different cabinets are the same as to length and width, varying only in height. The cabinet formed by interlocking may be 18¾ x 15¾; 18¾ x 11 or 18¾ x 5 or it may be a pyramid with the sections varying in width.

C221—Capacity 1500 Slides—18¾ x 11 x 3⅞
For Filing KODACHROME TRANSPARENCIES and 2 x 2" SLIDES

SPECIFICATIONS: All Paragon Tray Drawer Cabinets are made of reinforced steel construction, olive green finish. Interlocking device enables several units to be joined into one. Each sectional unit contains removable drawers with hand grip in front and rear. Interlocking steel base obtainable whenever required. **Constructed according to rigid specifications—not merely adapted.**

Address your orders and inquiries to Dept. P.
Manufactured Exclusively by

PARAGON C. & C. CO., Inc. • 2540 Belmont Ave., New York 58, N. Y.



in prothrombin time determinations

the test of patient safety

A dependable guide to anticoagulant dosage must give accurate, reproducible results with plasma from patients who are actually on anticoagulant therapy. Reagents standardized only against *normal* controls may not be accurate in the upper ranges of prothrombin time.

Simplastin (specially prepared thromboplastin-calcium) is rigidly standardized against both normal and dicoumarolized plasmas, whole and in dilution.

You can confirm the ease, accuracy and uniformity of Simplastin in your own laboratory. With the attached coupon you may obtain three 20-determination vials of Simplastin. Prepare them (*simply*, with only the addition of distilled water) and test each against the same plasma of a patient now on anticoagulant therapy.

Then repeat, using the same blood and three vials of any other thromboplastin reagent (old-style or newer type). Compare reproducibility for yourself, then judge Simplastin's ease of use, accuracy and dependability.

For the laboratory, Simplastin saves valuable time and effort . . . for the clinician, it provides a dependable and accurate guide.[1,2,3]

Supplied in boxes of 10: 6-determination or 20-determination vials.

1. Schilling, F. J.; De Natale, A., and Mottram, F. C.: Am. J. M. Sc. *222*:207 (Aug.) 1951. 2. Shapiro, S., and Weiner, M.: J. M. Soc. New Jersey *48*:1 (Jan.) 1951. 3. Shapiro, S., *et al.*: Am. Heart J. *40*:766 (Nov.) 1950.

Simplastin®

simple for the technician
accurate for the clinician

WARNER-CHILCOTT

Laboratory Supply Division
113 West 18th Street
New York 11, New York

Without obligation on my part, please send me three 20-determination vials of Simplastin.

NAME_____

INSTITUTION_____

ADDRESS_____

CITY_____ STATE_____

B

Reproducible prothrombin times

with Solu-Plastin®

(Thromboplastin Solution-Schieffelin)

. . . ACCURATE
. . . DEPENDABLE
. . . CONSISTENT

prothrombin activity determinations

"In our hands this [Solu-Plastin] has been found to be quite stable and yields relative uniform curves from lot to lot."

Pascale, L. R., and Olwin, J. H.: Circulation 9:230 (Feb.) 1954.

EVERY rigidly standardized lot (checked against both normal and dicumarolized human plasma) is ready for use . . . needs only to be mixed with calcium chloride solution as required . . . there is no waste. To assure sensitive, reproducible end points, Solu-Plastin may be used in any of the standard techniques . . . Quick one stage; or two stage; modified Owren.

Solu-Plastin retains full activity indefinitely at 4° C . . . for a minimum of 2 weeks at room temperature . . . somewhat shorter time at higher temperatures.

SUPPLIED: Bottles 10 cc (100 determinations) with like amount of 0.0125 M calcium chloride solution.

Easy-to-follow wall Directions Cards, useful Prothrombin Determination Records and full literature will be supplied on request. Write your name and address on the margin of this advertisement . . . return to:

Write also for the latest progress on Schieffelin's C·R·P·A® (C-reactive Protein Antiserum – Schieffelin) an aid in evaluation of anti-inflammatory therapy.

Schieffelin & Co. since 1794
Pharmaceutical and Research Laboratories
New York 3, N. Y.

A.M.A. Archives OF PATHOLOGY

Lint Granuloma

GEORGE AMROMIN, M.D.
OTTO SAPHIR, M.D.
and
IRENE GOLDBERGER, M.A., Chicago

Suture and other foreign-body granulomas occurring in surgical specimens are well described in the literature and are familiar to surgical pathologists. We have encountered a number of minute foreign-body granulomas due to "lint," a type which has received little attention. The lint in these instances was deposited in the skin during surgery or, in one instance, during or following injection with a hypodermic needle.

Lint, for the purpose of this discussion, is defined as the material obtained from fabric or gauze, particularly cotton, during its wear or use, either in ordinary circumstances or during surgical procedures. The latter is the type of lint which would be obtained from gauze or cotton during rubbing or surgical manipulation. It seems that a sponge usually referred to as "Zobec," which consists of a layer of cotton between folds of gauze, is particularly prone to shed lint particles into the tissues. Such granulomas would be of minor importance, if it were not for the fact that occasionally they may be responsible for the appearance of postoperative nodules or even abscesses and may be of medicolegal significance. An instance is described in which the presence of lint or fuzz, probably derived from cotton during the wiping of a hypodermic needle puncture wound, was in great part instrumental in clearing the issues involved in an instance of unexpected death.

Fragments of material obtained by scraping some cotton gauze were injected subcutaneously into rabbits. The tissue reaction to lint is described as seen in the subcutaneous tissue of the animals four days after its injection.

Submitted for publication Aug. 5, 1955.

This work was supported by the Ira Frank Fund.

From the Department of Pathology, Michael Reese Hospital. The department is in part supported by the Michael Reese Research Foundation.

APPEARANCE OF LINT PARTICLES IN BIOPSY SPECIMENS

From about 65 observations only a few pertinent instances may be cited.

CASE 1.—The specimen was taken from the upper eyelid of a 5-year-old boy eight months after a lid plastic procedure for ptosis. Within the subcutaneous tissue was abundant granulation tissue, consisting of fibroblasts, lymphocytes, large monocytes, and great numbers of multinucleated giant cells, of the foreign-body variety. In addition to what appeared to be remnants of absorbable suture material surrounded by giant cells, there were other relatively small giant cells, with varying numbers of nuclei, sometimes with only three to five nuclei, and containing minute refractile structures unstained in hematoxylin and eosin preparations and approximately 5μ in diameter. Some of these appeared in the form of a double half circle, between the arches of which a highly refractile slit was noted. This material could be clearly differentiated from silk particles and gut sutures.

CASE 2.—In a 2-day-old boy with atretic ileum an ileostomy was performed. Nine days later the atretic portions were resected, the terminal ileum was closed, and an ileocolostomy was performed. The serosa of the small intestine was covered with a thick layer of fibrin and polymorphonuclear leucocytes. Scattered over the serosa, in addition to this inflammatory exudate, were mononuclear cells and multinucleated cells of the foreign-body variety. In one area, surrounded by small foreign-body giant cells, there were minute or larger irregular refractile structures unstainable by the usual staining methods. They were smaller than those usually described in suture granulomas due to cotton and measured approximately 7μ in width and about 90μ in length. Often it was noted that two minute fibers were in close approximation and that a highly refractile slit was between them. In general, however, the fibers were of irregular size and were suggestive of cotton fibers.

CASE 3.—An 11-year-old Negro boy was admitted with a provisional diagnosis of poliomyelitis. Because of suggestive x-ray shadows about the hilus of the lung, a Kveim test for Boeck's sarcoid was performed. The biopsy specimen was from the test area.

Within the dermis about hair follicles and sweat glands and extending deeper into the underlying tissue and into the superficial portion of subcutaneous fat was a large amount of fibrous connective tissue, mononuclear cells, and giant cells of the foreign-body variety. The giant cells did not form any definite granulomas in association with fibroblasts or other inflammatory cells but were arranged and distributed in disorderly fashion. In one instance, however, there were epithelioid cells and (multinucleated) giant cells with abundant foamy cytoplasm but only two or three nuclei grouped about a central necrotic-appearing ovoid area. Within one giant cell there was a refractile structure about 30μ long and about 5μ wide. It had an irregular border and was not stainable by the usual routine staining methods. The majority of giant cells contained minute, curved, slightly refractile structures measuring about 7μ by 1μ or 2μ. Sometimes it seemed that between the curvature of the fibers a slit-like, refractile space was present. With hematoxylin and eosin stain, these were unstainable and appeared pale yellow or tan.

CASE 4.—The tissue was removed from an old incisional hernia of the anterior abdominal wall of a 60-year-old woman.

This specimen consisted of a hyalinized connective tissue, between the bundles of which were numerous foreign-body granulomas consisting of lymphocytes, a moderate amount of fibrous connective tissue, and foreign-body giant cells. Irregular structures were present between and within these giant cells. Many had sharp borders and did not resemble the previously described lint in granulomas. They closely resembled crystalline structures similar to talc granulomas. However, other small granulomas were also observed, consisting of small giant cells and inclusions resembling lint.

CASE 5.—In a 65-year-old man a biopsy specimen of a supraclavicular lymph node was taken from the same area as that from which a biopsy specimen had been taken one month previously. The clinical diagnosis was not determined. The patient had unexplained pleural effusion and weight loss.

There were large nerve bundles surrounded by fibroareolar tissue, small vessels, and foreign-body giant cells. Within the cytoplasm of some of these were thin refractile structures approximately 5μ in width and varying in length. The majority assumed a curvilinear appearance with highly refractile slit-like spaces. The giant cells, for the most part, were embedded in dense fibrous connective tissue.

CASE 6.—The tissue received was taken from an operative site for subdural hematoma. The original surgery had been done one month previously, at which time a hematoma and its wall were removed. However, there was gradual recurrence of symptoms, and the patient was reoperated upon, with a preoperative diagnosis of brain abscess.

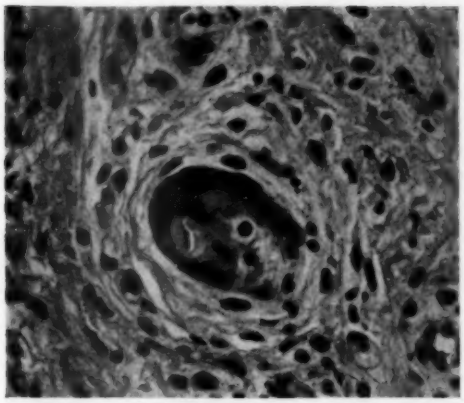


Fig. 1.—Giant cell with foreign body seemingly appearing as a curved empty space. Hematoxylin and eosin preparation; reduced about ⅓ from mag. × 400.

There were fragments identified as brain substance covered with abundant granulation tissue, fibroblasts, lymphocytes, and polymorphonuclear leucocytes. There were scattered multinucleated giant cells containing remnants of refractile structures, the majority of which were highly irregular, elongated or curved, and 5μ to 7μ wide. Some were up to 50μ long and more or less straight, while others were curved upon themselves and almost ovoid, with a slit-like refractile space in the ovoid structure. These were surrounded by the cytoplasm of foreign-body giant cells.

Thus, the outstanding and pertinent changes were either small granulomas or scattered multinucleated giant cells containing minute unstained refractile smaller or larger inclusions, which were often slightly curved. These were interpreted as minute fragments of gauze or cotton particles, fuzz or lint.

Lint is apparently shed from cotton, sutures, and gauze as well as from surgical sponges during their use. The fragments of lint in all specimens had a fundamentally similar appearance. Many were straight, 3μ to 7μ in diameter, and refractile. However, very characteristic was a hairpin-shaped appearance in which the material seemed to be bent sharply upon itself. In some instances the fragments formed an oval or a circle. Where the two arms were close together and parallel, the minute slit between them appeared refractile. This may well have been artifact resulting from air entrapment between the two arms of the foreign body during the histologic preparation. Regardless of its origin, this refractile zone was seen quite often in the lint granulomas. The younger granulomas showed greater numbers of polymorphonuclear leucocytes, as would be expected, whereas those which were older exhibited more and denser mature fibrous connective tissue. Only in lesions several days old were fragments seen lying free in tissue. In maturer or old granulomas they were exclusively and entirely within the cytoplasm of giant cells of the foreign-body variety.

Patients 1 and 6, we feel, had to be reoperated upon because of the lint which was deposited in the wound during the first operation. Although pus was obtained at surgery from Patient 6, it was sterile. There were, however, fragments of lint granulomas, which may well be blamed for the necessity for reoperation.

APPEARANCE OF LINT PARTICLES IN EXPERIMENTS

Surgical cotton gauze was scraped with a razor blade, and the resultant lint together

Fig. 2.—Two particles of lint in giant cells. Hematoxylin and eosin preparation; reduced about ⅓ from mag. × 435.

with tissue was embedded in paraffin and cut. The embedding of the material with tissue was done to facilitate its identification in the sections. The lint particles either did not stain with hematoxylin and eosin or were faintly pink in some instances. Fragments of lint were refractile and in every respect identical with those seen in the above-described foreign-body giant cells in the tissues removed from our patients. As would be expected, they varied considerably in size and shape. Some were straight and some curved. The ends of the fragments also varied in shape depending on the way they were cut during procurement for the experiment. The majority, in contrast to those which

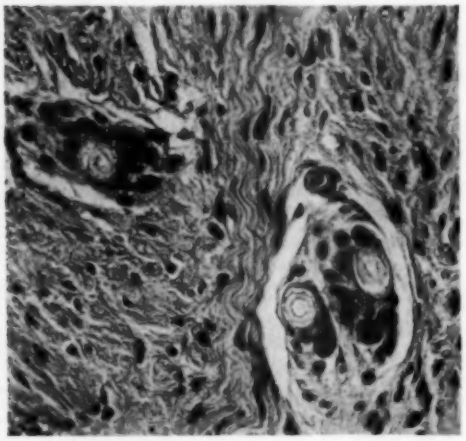


Fig. 3.—Three giant cells with lint, in experimental rabbit. Iron hematoxylin and eosin preparation; reduced about ⅛ from mag. × 400.

have been found in patients, had sharp angular ends and either were curved or formed ovoids, but in general were of similar size and shape to those seen in the tissue.

Lint particles were suspended in saline and injected subcutaneously into two rabbits. The skin was excised at the end of four days.

In addition to fibrosis and minute calcified foci of fat necrosis, there were fibroblasts and multinucleated giant cells of the foreign-body variety. The majority of the latter were in aggregates. Within some of these were characteristic refractile structures, approximately 5μ wide and identical with those described in

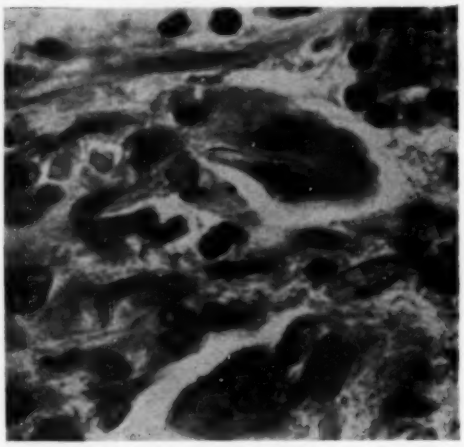
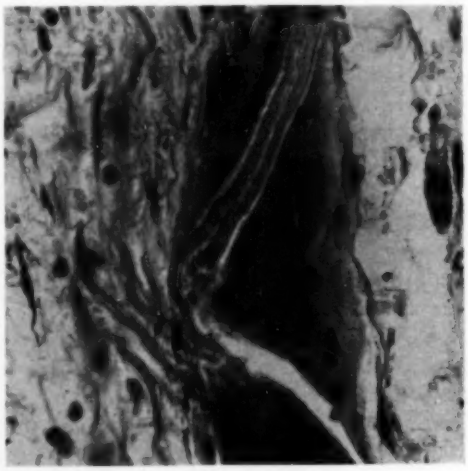


Fig. 4.—Lint in giant cell. Tissue shown in this Figure and Figure 5 was taken from a needle wound in the cubital region of an exhumed body. Hematoxylin and eosin preparation; reduced about ⅛ from mag. × 500.

human beings. Outspoken granulomas were not found.

From the foregoing, it seems clear that reaction to lint or fuzz left inadvertently in tissues is not rare. The reaction is principally a small accumulation of giant cells with a few nuclei and a lint particle in their center. Also outspoken granulomas may be present. Various inflammatory cells and fibrous connective tissue are often also encountered.

Fig. 5.—Large lint particle in giant cell. Hematoxylin and eosin preparation; × 500.



It must be realized that not all patients react in a similar fashion to lint; in some instances many giant cells with much granulation tissue and eventual fibrous scars may be found, and in others apparently only few of these elements are present, even though the amount of lint particles may be about the same. This holds, of course, also for the reaction to sutures and may well be governed by all the obscure principles involved in scar formation in general.

We have observed an instance in which the recognition of lint granulomas aided in the clarification of a medicolegal problem.* A 19-year-old youth was found dead in circumstances which would indicate that he was a drug addict. Also witnesses were found whose stories could be interpreted as branding him an addict. On court order the body was exhumed to determine the cause of death. There were two minute puncture holes in the cubital regions which could have been self-inflicted. However, dissection disclosed that one of these led into the antecubital vein, while the other ended blindly in the subcutaneous tissue. Serial sections of the subcutaneous tissue in this area disclosed typical lint granulomas as described above. It was thus clear that the recent puncture wounds must have been made by a needle which had been wiped or enclosed in gauze or cotton. Since it is improbable that a drug addict would use such a precaution, it was thought more probable that a professional medical worker had applied the needle with the usual technique and that the patient most likely had donated blood. In other words, the finding of giant cells containing lint particles provided evidence to rule out self-inflicted needle punctures for addiction purposes.

The presence of lint particles in smaller branches of the pulmonary arteries has been studied in detail by Von Glahn and his co-workers † and was further described by Konwaler.[2] Wiping the intravenous needle with gauze before insertion into a vein apparently is sufficient to deposit lint, which then gains access to the circulation. A similar process of drawing cotton sutures through subcutaneous tissues may also result in such deposition. Merely sponging an open wound with cotton gauze, even with the small brain sponges as in Case 6, may be sufficient to deposit enough lint to cause postoperative complications. The illustrations of the cotton fragments (lint) in Konwaler's article as well as those produced experimentally by Von Glahn and his co-workers,‡ except for the intravascular location, are identical with ours.

SUMMARY

The importance of lint as found in postoperative and needle puncture wounds is emphasized. Morphologic identification of the material in tissue sections and the reproduction of the lesion in an animal are illustrated and described. Seven instances in which lint was discovered in postoperative wounds or after hypodermic injection, presumably after the needle or the wound had been wiped with gauze, are described, and the ease is emphasized with which lint may be shed from cotton, linen sutures, and gauze sponges. In rare instances, lint deposition in tissues, particularly during brain surgery, may be of serious consequence.

REFERENCES

1. Von Glahn, W. C., and Hall, J. W.: Reaction Produced in the Pulmonary Arteries by Emboli of Cotton Fibers, Am. J. Path. **25**:575-595, 1949.

2. Konwaler, B. E.: Pulmonary Emboli of Cotton Fibers, Am. J. Clin. Path. **20**:385-389, 1950.

3. Von Glahn, W. C.; Hall, J. W., and Sun, S.-C.: Arteritis in Guinea-Pigs, Produced by Emboli of Cotton, Resembling the Arteritis of Hypersensitivity, Am. J. Path. **30**:1129-1139, 1954.

* Dr. Edwin F. Hirsch prepared the pertinent slides.

† References 1 and 3.

‡ References 1 and 3.

The Pathologic Effects of Smoking on the Larynx

ROBERT F. RYAN, M.D.
JOHN R. McDONALD, M.D.
and
KENNETH D. DEVINE, M.D., Rochester, Minn.

If tobacco is harmful to the larynx, then some pathologic changes should be present in the larynx of excessive smokers that are not present in the nonsmoker. In an often-quoted article on the "smoker's larynx" Myerson [1] described congestion and edema that lead to fibrosis and myxomatous changes. Some authors have written about the irritating effects that various hygroscopic agents commonly used in tobacco have on the throat. Others * have written of an increasing incidence of carcinoma of the larynx and a frequent association between carcinoma and excessive smoking. Hoarseness is often ascribed by laryngologists to chronic irritation, and heavy smoking is high on the list of causes of chronic irritation. Many authors † have considered smoking a major cause of keratosis of the larynx. Thus, while much has been written about smoking and the larynx from a clinical standpoint and from the standpoint of changes in selected patients, we have been unable to find any articles in which pathologic differences between the larynx of the heavy smoker and that of the nonsmoker have been described on the basis of postmortem examination. Hence, we undertook the present study to see whether any such differences could be demonstrated.

The over-all plan was first to make a pilot study in which we would compare slides made from the larynx of excessive smokers with those made from nonsmokers, the latter group being normal by definition. If any consistent histologic differences should be found between the two groups, then the second part of the study would be undertaken. In this part, sections would be made from the larynx of subjects whose smoking habits were unknown to us at the time of examination of the slides, and the sections would be separated into two groups: (1) those in which the laryngeal epithelium appeared normal or comparable to that of the nonsmokers in the pilot study and (2) those in which the laryngeal epithelium did not appear normal; that is, it appeared to be comparable to that of the excessive smokers in the pilot study. The attainment, in the second part of the study, of a fair degree of correlation between our findings and the reported smoking habits of the subjects would be confirmation of the validity of the findings of the pilot study.

Submitted for publication July 13, 1955.

Read before the Section on Pathology and Physiology at the 104th Annual Meeting of the American Medical Association, Atlantic City, N. J., June 7, 1955.

Abridgment of thesis submitted by Dr. Ryan to the Faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of Master of Science in Surgery.

Fellow in Surgery, Mayo Foundation (Dr. Ryan); Section of Surgical Pathology (Dr. McDonald), and Section of Plastic Surgery and Laryngology (Dr. Devine), of the Mayo Clinic and Mayo Foundation. The Mayo Foundation is a part of the Graduate School of the University of Minnesota.

* References 2 through 6.

† References 3, 4, and 6.

PART 1. PILOT STUDY

Procedure.—Since the pathologic effect of smoking was the factor to be evaluated in this part of the study, the cases had to be as nearly alike as possible except for the factor of smoking. The larynxes used were chosen from some 1500 that had been removed at necropsy and preserved. The factors of growth and degeneration were avoided by using specimens from subjects roughly in the age group from 40 to 60 years. Possible sexual differences were eliminated by restricting the study to men. A specimen was not used if the clinical record mentioned any history of hoarseness or recent complaints referable to the larynx. The occupation was checked to rule out undue vocal strain. Possible pathologic changes due to intratracheal anesthesia [7] or bronchoscopy were ruled out in all cases. A specimen was not used if the body was suspected of being the site of any granulomatous process that might remotely have affected the larynx. A final safeguard was to use the larynx from patients who had died a relatively sudden death and had not subjected the larynx to possible trauma or drying or crusting effects due to coma.

The history of smoking or nonsmoking contained in the clinical record was confirmed by a letter sent to the next of kin, in which they were asked about the type and amount of tobacco the subject had used and the length of time he had used it.

The subjects were classified according to the method of Wynder and Graham [8] (Table 1). Because of the rigid criteria used, only three nonsmokers and nine smokers were found to be suitable among the 1500 cases

TABLE 1.—*Classification of Subjects According to the Method of Wynder and Graham*

	Group	Cigarettes per Day for More Than 20 Yr.*
0	Nonsmoker	Less than 1
1	Light smoker	1 to 9
2	Moderate-to-heavy smoker	10 to 15
3	Heavy smoker	16 to 20
4	Excessive smoker	21 to 34
5	Chain smoker	35 or more

* 1 cigar = 5 cigarettes; 1 pipeful = 2½ cigarettes.

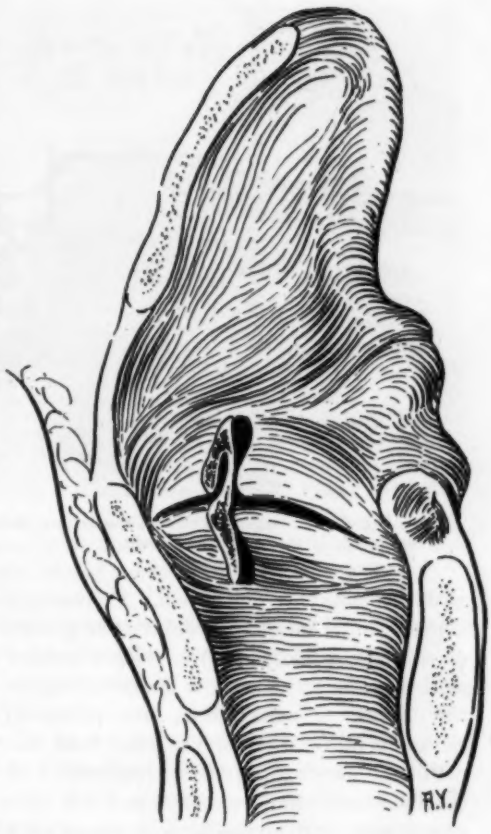


Fig. 1.—Lateral view of the larynx showing the site from which the tissue used in this study was removed.

available. Of the smokers, one man smoked several cigars a day and each of the others smoked at least one package of cigarettes a day; hence, they would all be classified as heavy or excessive smokers.

Each larynx was opened posteriorly and examined, and no gross abnormalities were noted. Figure 1 shows the site from which a section was taken from each half of the larynx near the junction of the anterior and middle thirds of the true cord; it also shows the false cord, ventricle, true cord, and subglottic mucosa. In taking the sections great care was used to make the cuts perpendicular to the surface, and a straight razor was used to avoid undue trauma and distortion. Sections made from these blocks were stained

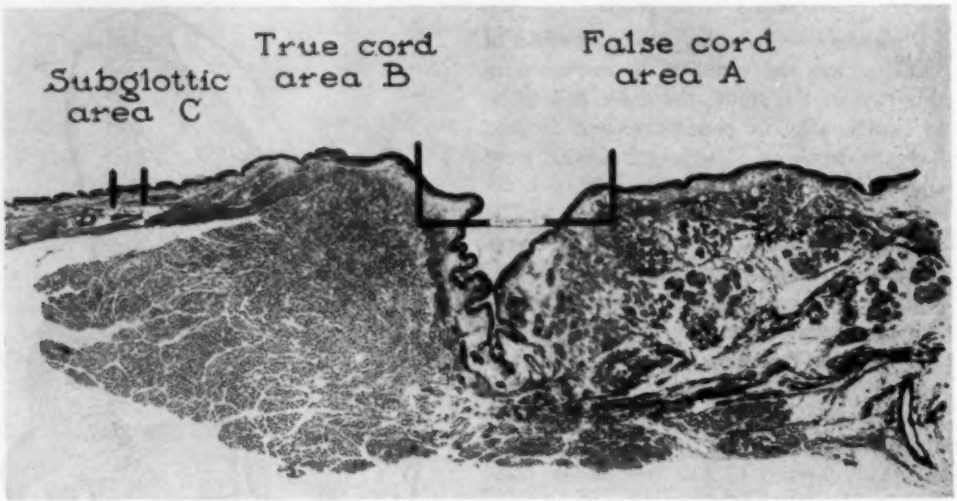


Fig. 2.—Enlarged section of the larynx showing the sites where the epithelial thickness was measured. Hematoxylin and eosin.

with hematoxylin and eosin. Preliminary comparison of the slides from the two groups of subjects disclosed (1) a thicker surface epithelium in the larynx of smokers than in the larynx of nonsmokers, due partly to excess keratinization of the true cord but mainly to varying degrees of hyperplasia of the false cord, the true cord, and the subglottic area, with epithelial pegs, larger cells and more prominent and deeply stained nuclei giving a more active appearance to the epithelium of the smokers as compared with the thin and somewhat atrophic-appearing epithelium of the nonsmokers; (2) a greater degree of round-cell infiltration in the smokers; (3) slightly more edema in the larynx of smokers, and (4) squamous-cell metaplasia of the surface epithelium of the false cord and occasionally of the subglottic area in the smokers but not in the nonsmokers. In situ lesions or other evidence of malignancy was not seen in any of the

Fig. 3.—Section of the subglottic mucosa from Case 2 (a nonsmoker) of the pilot study. Hematoxylin and eosin; × 50.

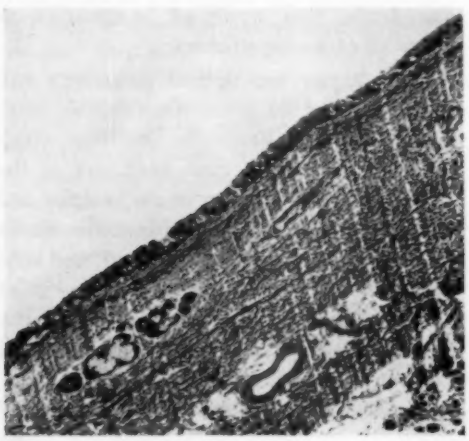
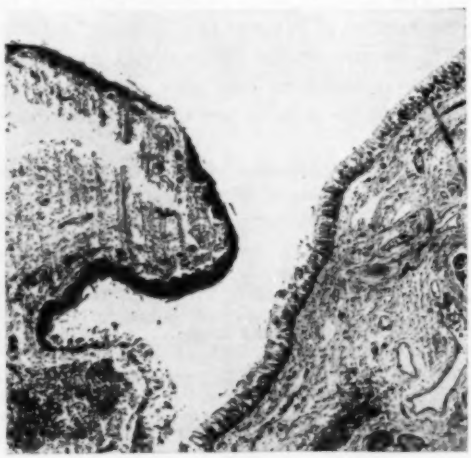


Fig. 4.—Section of the true cord and the false cord from Case 2 of the pilot study. Hematoxylin and eosin; × 50.



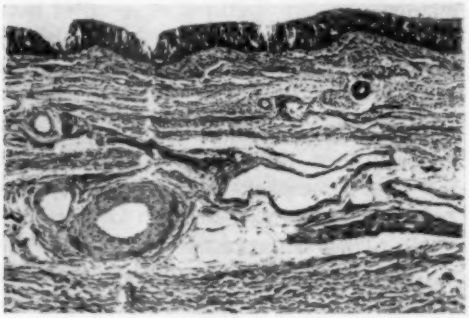


Fig. 5.—Section of the subglottic mucosa from Case 4 (an excessive smoker) of the pilot study. Hematoxylin and eosin; × 50.

Fig. 6.—Section of the true cord and the false cord from Case 5 (an excessive smoker) of the pilot study. Hematoxylin and eosin; × 50.

slides from either the nonsmokers or the smokers.

Obviously most of these changes are matters of degree and are subject to varying interpretation; an exception is the thickness of the epithelium, which can be measured objectively with fair accuracy. Hence, the next procedure was to measure the thickness of the epithelium. Since the epithelium varied in thickness in different areas of each slide, standard areas had to be chosen on each slide for comparison. To do this objectively each slide was viewed macroscopically, since the thickness of the epithelium could not be seen when viewed in this manner, and then, as shown in Figure 2, three segments were designated in which the thickness of the epithelium was to be measured: The first two were on the false cord and on the most prominent portion of the tip or free edge of the true cord respectively, and the third was down about 1 cm. on the tracheal side. Each segment was approximately 2 or 3 mm. long. Next, the maximal, minimal, and average thicknesses of the epithelium in each of the three segments were measured

TABLE 2.—*Pilot Study—Thickness of the Epithelium of the Larynx in Nonsmokers and Smokers*

Case	Age, Yr.	Cigarettes Used: Packages per Day	Cigarettes Used: Years Used	Thickness of Epithelium, μ: False Cord Av.	False Cord Max.	False Cord Min.	True Cord Av.	True Cord Max.	True Cord Min.	Subglottic Area Av.	Subglottic Area Max.	Subglottic Area Min.
Nonsmokers												
1	65	..	..	75	90	60	45	60	30	37.5	45	30
2	50	..	..	75	90	60	52.5	60	45	60	..	..
3	52	..	..	75	105	45	48.8	90	30	41	45	30
Average, nonsmokers	55.7	..	..	75	..	..	48.8	..	..	46.2	..	..
Smokers												
4	47	1	35	90	105	75	71	120	45	82	165	45
5	47	Heavy	15+	90	150	60	105	300	45	81	225	30
6	45	1	27	84	..	..	60	75	30	60	90	60
7	38	Heavy (cigars)	..	96	120	75	60	105	30	70	105	60
8	64	1-1½	"Years"	83	135	75	83	165	45	75	..	..
9	57	1½	35	91	150	45	60	150	45	67	75	45
10	51	1-1½	"Years"	84	..	..	90	130	45	82	..	..
11	61	1+	20+	86	150	60	75	135	45	75	90	60
12	57	1½	..	113	180	105	75	..	..	75	..	..
Average, smokers	51.6	..	..	90.8	..	..	75.4	..	..	71.8	..	..

with a micrometer. The same microscope with a ×10 ocular and a ×10 objective was used for the entire study.

Results.—Figure 3 shows a section of the epithelium in the subglottic area, and Figure 4 shows the tip of the true cord and part of the false cord, both sections being from a nonsmoker. The thinness of the epithelium is to be noted. Figure 5 shows the epithelium in the subglottic area, and Figure 6 shows the true cord and false cord, both sections being from an excessive smoker. The beginning squamous metaplasia in the false cord and the excess formation of keratin in the true cord are to be noted in addition to the increased thickness of the epithelium.

Table 2 summarizes the information on smoking habits and thickness of the epithelium in the individual nonsmokers and smokers of the pilot study.

TABLE 3.—*Pilot Study—Comparison of Thickness of Epithelium in Smokers and Nonsmokers*

Group	No.	Mean Thickness of Epithelium, μ		
		False Cord	True Cord	Subglottic Area
Nonsmokers	3	75.0	48.8	46.2
Smokers	9	90.8	75.4	70.8
Difference	..	15.8*	26.6*	24.6†

* Difference statistically significant by t test, level <0.05.
† Difference statistically significant by t test, level <0.01.

Table 3 shows that the difference between the mean thickness of the epithelium of the nonsmokers and that of the smokers has statistical significance. These differences perhaps have even more significance when we consider that the average age of the smokers was 51.6 years as compared with 55.7 years for the control group, and that the epithelium of the larynx becomes thicker with age, according to Eggston and Wolff.[9]

In evaluating the results of the pilot study, one must ask, "What is the normal thickness of the epithelium of the larynx of men in the general age group studied, namely, approximately 40 to 60 years?" None of nine standard textbooks of histology and of ear, nose, and throat histopathology that were consulted gave any actual measurements in microns. In fact, pictures of so-called normal epithelium appeared in some cases to be similar to that seen in excessive smokers in our study.

For the purposes of the present study the appearance of the laryngeal epithelium of the three nonsmokers of the pilot study was regarded as normal for men of this age group. Thus, if sections of a larynx showed varying degrees of the four changes noted in the smokers in the pilot study, they were considered deviations from the normal.

PART 2. STUDY OF A RANDOM SERIES OF SUBJECTS

Procedure.—Sixty larynxes that had been removed at necropsy in the previous two years were obtained. When they were first obtained, we knew only that each larynx was from a man in the approximate age group of 40 to 60 years. Fifty of the larynxes were found to be normal on macroscopic examination, and sections of these were made as in the pilot study. These slides were labeled with only the necropsy number. The thickness of the epithelium was measured as in the pilot study. One of us (Ryan) studied each slide and then recorded an opinion as to whether this was a normal-appearing epithelium as found in the cases of nonsmokers of the pilot study or whether it deviated from the normal as indicated by various histologic changes such as were found in the excessive smokers in the pilot study. In some cases one could say unequivocally, "Yes, this is a normal laryngeal epithelium," and in other cases, "No, this is not a normal-appearing laryngeal epithelium." These opinions were recorded as "yes" and "no," respectively. In other cases the estimate was equivocal and was recorded as "?". In still others there was some doubt, but an estimate was ventured and recorded as "?yes" or "?no." Next, the other two of us (McDonald and Devine) again reviewed the slides from the pilot study, and then independently reviewed all the slides from the random series, and recorded an opinion as to whether or not the epithelium was normal. This means, in effect, that if a section was recorded as

being normal the observer judged that the subject had not been a smoker, but if a section was recorded as not being normal the observer judged that the subject had been a smoker.

In the meantime letters of inquiry about the smoking habits of the subjects had been sent to the next of kin, and the replies to these and the clinical records were used to learn the actual smoking habits of the subjects. In 10 cases there was a discrepancy between the smoking history given in the clinic record and the answers supplied by the next of kin; for example, one would report that the subject used a half package of cigarettes per day, while another would report that he used one package per day. These 10 cases were dropped, therefore, because of the indicated inaccuracy of the information relative to smoking. Thus, a reliable answer was found for only 40 of the 50 subjects, and the results for Part 2 of the study are based upon these 40 subjects.

TABLE 4.—*Random Series—Mean Thickness of Laryngeal Epithelium in Relation to Smoking Habits of Subjects*

Group	No.	Mean Thickness of Epithelium, μ *		
		False Cord	True Cord	Subglottic Area
Nonsmokers	9	76.9±11.5	66.7±12.0	66.7± 5.2
Light-to-moderate smokers	5	84.0± 6.5	64.5±14.2	69.0±11.2
Heavy-to-excessive smokers	26	84.6± 3.6	79.9± 6.9	75.6± 4.3

* The figure after the ± is the standard error of the mean.

For purposes of analysis the 40 subjects were divided according to the known histories into three groups, namely, nonsmokers, light-to-moderate smokers, and heavy-to-excessive smokers. The answers given by two of the three of us were used as our answer when there was any disagreement among us.

Results.—Table 4 shows the mean thickness of the laryngeal epithelium in relation to the smoking habits of the subjects in the random series. It is apparent that although the difference in mean epithelial thickness in the three groups is not statistically significant, the thickness shows generally an increase with increase of amount of smoking in the groups concerned.

In six of the nine nonsmokers the epithelium was judged normal by the three of us. One error was in a case in which roentgen rays had been applied to the lymph nodes of the neck in the treatment of metastatic growths from a cancer of the tongue; this treatment may have accounted for the thickened epithelium. Not counting this case, the correlation between histologic appearance and smoking habits was high; that is, six of eight nonsmokers were correctly designated.

The poorest correlation between histologic appearance and smoking habits was in the group of light-to-moderate smokers. Of the five subjects in this group, three were judged to have normal-appearing laryngeal epithelium. Two of these three, however, were cigar smokers, who usually do not inhale.

Of the 26 heavy or excessive smokers, 4 were judged to have normal-appearing epithelium. In three of the four there was no agreement among us, and in the fourth a pipe and cigars were smoked.

The Section of Biometry and Medical Statistics of the Mayo Clinic analyzed these data and found that our designation of the cases as normal or as deviations from normal, based on the four criteria found in the pilot study, correlated with the actual smoking habits of the subjects for each of us individually, and for all three collectively, to a degree that has statistical significance.

COMMENT

It should be remembered that the opinions given by the three of us regarding deviations from normal were based on the four criteria described in the pilot study and not just on the thickness of the laryngeal epithelium. In this regard it is to be noted that the difference in mean epithelial thickness for nonsmokers and smokers in the random series (Part 2 of the study), shown in Table 4, does not have statistical significance; this is in contrast to the difference found in the pilot study, which does have statistical significance.

This apparent discrepancy can be largely explained on the basis of two factors: first, the increased thickness of the laryngeal epithelium of the smokers in the pilot study represents the maximal effects of smoking, since all other causes of trauma or chronic irritation to the larynx were ruled out and these subjects were specifically chosen because of their excessive smoking habits; and, second, other factors of trauma or chronic irritation were not ruled out in the subjects of Part 2 (random series), and thus the nonsmokers of Part 2 had a much thicker epithelium than did the nonsmokers of the pilot study. Since our base line for normality was much higher in Part 2, the difference between the nonsmokers and smokers in this part had to be correspondingly less. While the difference in mean epithelial thickness for nonsmokers and smokers in Part 2 does not have statistical significance, we believe that if enough subjects were studied and if the variations we have found were constant, the difference between nonsmokers and smokers would then be found to be significant statistically.

In the study as a whole two major limitations should be considered. The first is the small number of cases. As noted previously, the three nonsmokers and the nine smokers of the pilot study constitute a small number of subjects on which to base conclusions, even though the results may have statistical significance. Second, since necropsy specimens were used and the next of kin could not be interviewed personally, the study is subject to the limitations imposed by follow-up letters.

In considering the thesis that smoking causes pathologic changes in the larynx, one must remember certain points. The basic anatomy of the larynx is the same in the smoker as in the nonsmoker, and presumably the functions are the same. The false cord forms a zone of slight constriction, below which the laryngeal ventricle forms a somewhat wider zone. Below the ventricle the true cord again forms an area of constriction for the air column as it passes down into the trachea. The cross-sectional area of the air column is greatly increased once it passes the true cord and enters the subglottic area of the larynx. The diameter of the passageway is then relatively constant until the bifurcation of the trachea is reached.

If it is assumed for the moment that there is some substance or agent in smoke that can cause the pathologic effects or differences noted in the pilot study, how and why does such a substance act on the larynx? The work of Proetz [10] gives a logical answer. He built a machine that would smoke cigarettes in much the same manner that a person smokes them and learned many basic principles about the behavior of smoke. He found that "inhaled" smoke blown through a ¼ in. (0.64 cm.) tube onto a piece of white paper would not stain it, but that smoke blown through a similar tube the tip of which had been drawn out into a capillary would produce a fairly heavy stain on the paper.

Proetz also found that the blowing of smoke through a kinked tube caused tar to be deposited upon the wall of the tube distal to the kink. Similarly, if the tube was drawn out in the middle, the deposition occurred just beyond the narrow point. It was here that the narrow column of smoke expanded, eddy currents were set up, the rapid forward motion was lost, and the suspended particles were precipitated out. Proetz said that smoke consists of tarry substances present as minute globules, that it is heavier than air and can be poured as a liquid, and that it settles out on standing. In experiments on animals he found that blowing smoke through the nostrils caused the tars to be deposited on the septum and turbinates just beyond the narrow point of the meatus. He also found that these tars stopped the action of the cilia wherever they were deposited.

On the basis of this information, it seems that after tobacco smoke passes through the slight constriction at the false cord a portion of its tars should be deposited on the true cord and in the laryngeal ventricle, and that after the smoke passes through the narrow opening of the true cord a portion of its tars should be deposited in the subglottic area.

As a corollary to the results of the present study, there is abundant evidence that histologic changes of epithelium similar to those observed in our study can be produced in animals. Brosch, Wacker and Schmincke, and Hoffman and co-workers are all quoted by Friedell and Rosenthal [11] as having produced epithelial proliferation and thickening by the application of tobacco tars in experimental animals. Helwig [12] was able to produce epithelial hyperplasia in animals with tobacco tars. Flory,[13] as well as other workers, was unable to confirm Ruffo's work on the production of malignant lesions, but he produced papillomas and carcinomatoid tumors in rabbits with different distillates of tobacco tars. The carcinomatoid tumors showed invasive hyperkeratosis but no metastasis. Kreshover [14] in 1952 reported that blowing smoke on the lips and ears of mice produced mostly hyperplasia of the epithelium and edema of the subcutaneous tissue. He found that the effect of tobacco smoke was greatly lessened in castrated animals. Wynder and co-workers [15] recently produced skin cancers in one strain of mice by the application of cigarette tars. As with all experiments on animals, it is not proper to apply all of this information with great certainty to man. Moreover, there is no agreement in the literature as to which one of the substances present in smoke or tobacco tar is responsible for the pathologic effects seen in the present study or in the animal experiments.

In man, clinical experience shows that smoking has known effects in other areas that are analogous to the observed effects on the larynx. Cummer [16] reported that certain types of leucoplakia can be produced in the nonsmoker by heavy smoking and pointed out that such lesions are found in heavy smokers only in the areas not covered by partial dentures. Vaughan [17] cleared up certain types of palatal leucoplakia by having the patients wear vulcanite plates. The information on leucoplakia may be summed up as follows: Many authors [18] in writing about leucoplakia and its relationship to oral malignant transformation have expressed the belief that tobacco is the chief irritant that causes leucoplakia of the mouth, the abnormality being an epithelial thickening due to hyperplasia with varying degrees of hyperkeratosis and cellular infiltration. Moreover, the lesions can often be cured or made to disappear by cessation of the use of tobacco, or by covering the lesions with a vulcanite plate when they occur on the palate. Such information supports the thesis that smoking can cause the lesions of the larynx observed in the present study.

Clinical experience also indicates that tobacco is probably responsible for localized areas of keratosis or leucoplakia as shown on biopsy of the larynx. Cases ‡ have been described in which the lesions cleared up with cessation of smoking, only to reappear with the resumption of smoking.

SUMMARY

Comparison of microscopic sections from the larynx of nonsmokers with sections from the larynx of known excessive smokers disclosed (1) a thicker surface epithelium in smokers than nonsmokers due in part to excess keratinization of the true cord but mostly to epithelial hyperplasia at all three sites measured, namely, the false cord, the true cord, and the subglottic area; (2) a greater degree of round-cell infiltration in the smokers; (3) slightly more edema in the smokers, and (4) the presence of squamous-cell metaplasia in only the excessive smokers in this study.

In the second part of the study we took a random series of male subjects in the approximate age group from 40 to 60 years and used the foregoing criteria to designate the larynxes as normal or as not normal, "normal" being defined as the appearance of the larynx seen in the nonsmoker of the pilot study. The designations correlated well with the actual smoking habits of the subjects.

This evidence supports the thesis that excessive smoking is associated with pathologic changes in the larynx.

‡ References 4 and 6.

REFERENCES

1. Myerson, M. C.: Smoker's Larynx: Clinical Pathological Entity, Ann. Otol. Rhin. & Laryng. **59**:541-546 (June) 1950.

2. Clerf, L. H.; Putney, F. J., and O'Keefe, J. J.: Carcinoma of the Larynx, Laryngoscope **58**:632-641 (July) 1948.

3. Kennaway, E. L., and Kennaway, N. M.: Further Study of the Incidence of Cancer of the Lung and Larynx, Brit. J. Cancer **1**:260-298 (Sept.) 1947.

4. Miller, A. H., and Fisher, H. R.: Carcinoma-in-Situ of the Larynx, Ann. Otol. Rhin. & Laryng. **62**:358-370 (June) 1953.

5. Valko, P.: Smoking and the Development of Malignant Tumours of the Larynx, Československ. otolaryng. Praha, **1**:102-105, 1952.

6. Wallner, L. J.: Smoker's Larynx, Laryngoscope **64**:259-270 (April) 1954.

7. Smiley, W. A.: Polypoid Granuloma of Larynx Following Endo-Tracheal Anesthesia, Ann. Otol. Rhin. & Laryng. **49**:556-558 (June) 1940.

8. Wynder, E. L., and Graham, E. A.: Tobacco Smoking as a Possible Etiologic Factor in Bronchiogenic Carcinoma: Study of 684 Proved Cases, J. A. M. A. **143**:329-336 (May 27) 1950.

9. Eggston, A. A., and Wolff,. D.: Histopathology of the Ear, Nose and Throat, Baltimore, Williams & Wilkins Company, 1947, p. 857.

10. Proetz, A. W.: Some Preliminary Experiments in the Study of Cigarette Smoke and Its Effects upon the Respiratory Tract, Ann. Otol. Rhin. & Laryng. **48**:176-194 (March) 1939.

11. Friedell, H. L., and Rosenthal, L. M.: Etiologic Role of Chewing Tobacco in Cancer of Mouth: Report of 8 Cases Treated with Radiation, J. A. M. A. **116**:2130-2135 (May 10) 1941.

12. Helwig, F. C.: Influence of Tobacco and Other Extracts on the Epithelial Cell, J. Kansas M. Soc. **27**:37-40 (Feb.) 1927.

13. Flory, C. M.: Production of Tumors by Tobacco Tars, Cancer Res. **1**:262-276 (April) 1941.

14. Kreshover, S. J.: Effect of Tobacco on Epithelial Tissues of Mice, J. Am. Dent. A. **45**:528-540 (Nov.) 1952.

15. Wynder, E. L.; Graham, E. A., and Croninger, A. B.: Experimental Production of Carcinoma with Cigarette Tar, Cancer Res. **13**:855-864 (Dec.) 1953.

16. Cummer, C. L.: Leukoplakia (Leukokeratosis) of the Palate, Papular Form: Its Relation to the Use of Tobacco, J. A. M. A. **132**:493-498 (Nov. 2) 1946.

17. Vaughan, H. C.: Leukoplakia, J. Am. Dent. A. **22**:65-66 (Jan.) 1935.

18. Moore, G. E.; Bissinger, L. L., and Proehl, E. C.: Tobacco and Intraoral Cancer, S. Forum (1952) pp. 685-688, 1953.

Inborn Errors of Lipid Metabolism

Clinical, Genetic, and Chemical Aspects

DAVID ADLERSBERG, M.D., New York

This study on inborn errors of lipid metabolism deals with two errors of lipid metabolism, idiopathic hyperlipemia and idiopathic hypercholesteremia. Other disturbances of lipid metabolism such as sphingomyelin lipidosis (Niemann-Pick's disease) or cerebroside lipidosis (Gaucher's disease) are not included in this presentation. It was pointed out previously that a close relationship exists between idiopathic hypercholesteremia (familial xanthomatosis) and atherosclerosis.* The data of this study suggest that idiopathic hyperlipemia represents a similar predisposing factor for atherosclerosis.†

Idiopathic hyperlipemia differs from idiopathic hypercholesteremia in the milky or creamy appearance (lactescence) of the serum and in decided elevation of serum neutral fats and total lipids in the fasting state. Although both abnormalities resemble each other to a certain extent, there are distinct clinical and biochemical differences between them.

Submitted for publication July 13, 1955.

From the Departments of Medicine and Chemistry of The Mount Sinai Hospital.

Read before the Section on Pathology and Physiology at the 104th Annual Meeting of the American Medical Association, Atlantic City, N. J., June 7, 1955.

This investigation was supported in part by research grants H-564 and H-982 of the National Institutes of Health, United States Public Health Service.

Dr. C. Wang and Dr. E. T. Bossak assisted in the lipid and lipoprotein analyses. Miss Inge Manpel and Miss Maria T. Torres gave technical help.

* References 1 through 5.

† References 6 through 8.

IDIOPATHIC HYPERLIPEMIA

Twenty Families and Five Additional Persons with Idiopathic Hyperlipemia.—During the past few years we have encountered 25 persons (index patients) with idiopathic hyperlipemia and have studied 84 members of the families of 20 of these patients. Together with the 5 patients with idiopathic hyperlipemia whose families were not available for study, this group includes 89 persons, 58 male and 31 female.

All of the 20 probands, 3 of their close relatives, and the 5 individual patients exhibited the characteristic sign of lactescence of the serum in the fasting state. The concentration of serum total lipids among the 25 index patients ranged from 1070 to 12,400, average 3598, mg. per 100 ml.; that of the neutral fats averaged 2387 mg. per 100 ml. The concentration of serum cholesterol among the index patients ranged from 213 to 1480, average 623 mg., per 100 ml.; that of the phospholipids, 188 to 1244, average 588, mg. per 100 ml. The average levels for the entire series were: For serum total lipids, determined for 48 persons, 3373 mg. per 100 ml.; for neutral fat 2541 mg. per 100 ml.; total cholesterol, analyzed in 60 persons, 412 mg. per 100 ml., and for esterified cholesterol 260 mg. per 100 ml. The latter fraction amounted thus to 63% of the total serum cholesterol. Serum phospholipids averaged 420 mg. per 100 ml. The cholesterol: phospholipid ratio was 0.98.

In the former study we selected a level of 300 mg. per 100 ml. of total serum cholesterol by the Bloor method as indicative of hypercholesteremia. On the basis of additional data, figures above 280 mg. per 100 ml. by the Sperry-Schoenheimer method may be considered abnormal.[2] Based on a similar conservative estimate, serum phospholipids (Sperry's modification of the Fiske-SubbaRow method) above 320 mg. per 100 ml. and total lipids (Bloor's method) above 1200 mg. per 100 ml. may indicate abnormal elevation of the respective frac-

Fig. 1.—Xanthoma tuberosum in a patient with idiopathic hyperlipemia.

tion. Neutral fat was calculated by subtracting the figure for total cholesterol plus phospholipid from the value for total lipid.

In the entire series of 89 persons, xanthelasma of the eyelids was present in 2; xanthoma tendinosum was seen only once, whereas xanthoma tuberosum was found in 12 persons. Coronary artery disease was present in 30 persons of the series; among the 25 index patients it was encountered in 14, and among their family members, in 16. In contrast to the next series of patients with idiopathic hypercholesteremia, most patients of this series sought treatment for noncardiac conditions. Five of the index patients had symptoms and signs of relapsing pancreatitis. Six had mild diabetes mellitus. Four of the index patients had no stigmata of the disturbance in lipid metabolism, and two others were asymptomatic except for the presence of hepatomegaly and hepatosplenomegaly, respectively.

The data indicate that the most frequently encountered abnormalities in this series were lactescence of the serum with elevation of total lipids and neutral fats, hypercholesteremia, and coronary atherosclerosis. Xanthoma tuberosum was next in frequency, while xanthelasma and xanthoma tendinosum were seen least often. Relapsing pancreatitis and diabetes were each present in about one-fifth of the index patients. Hypertension was infrequently encountered. It was present in only four of the index patients and in seven of their family members.

A typical lesion of xanthoma tuberosum is shown in Figure 1. The presence of such lesions should alert the physician to study the patient and his family for evidence of idiopathic hyperlipemia and for heart disease.

IDIOPATHIC HYPERCHOLESTEREMIA

Seventy-Seven Families and Forty-Nine Additional Persons with Idiopathic Hypercholesteremia.—Our previous group included 64 persons (index patients) with this disorder and 137 members of the families of 35 of these patients.[2] This "old" group comprised, then, 172 persons in the families and 29 individual patients whose families were not available for study, a total of 201 persons. Since then an additional group of 62 persons (index patients) have been studied and 127 family members of 42 of these patients. Thus, the "new" group comprised 169 persons in the families and 20 individual patients, a total of 189 persons. Adding the "old" and the "new" groups, the series reported here included 77 index patients with 341 family members and 49 individual patients, to total 390 persons, 224 male and 166 female.

The serum of these persons was without exception clear when examined in the fasting state. The serum cholesterol level was determined in 317 of the 390 persons. A total of 223 (70%) had hypercholesteremia (300 mg. per 100 ml. or above by the Bloor method, or 280 mg. per 100 ml. or above by the Sperry-Schoenheimer method). The study of the serum lipids in the "new" group was extended to the complete serum lipid partition. The total serum cholesterol averaged 366 mg. per 100 ml. for the 62 index patients and 319 mg. per 100 ml. for 80 persons of the entire group. Esterified cholesterol averaged 263 and 231 mg. per 100 ml., respectively. Esterified cholesterol amounted to 67% of the total serum cholesterol. The serum phospholipid concentration for 57 of the index patients averaged 342 mg. per 100 ml., and for 70 persons of the entire group 314 mg. per 100 ml. The cholesterol phospholipid ratio was 0.9 and 1.0, respectively. The concentration of serum total lipid averaged 1144 mg. per 100 ml. for 52 of the index patients and 1101 mg. per 100 ml. for 76 persons of the entire group; that of neutral fat 436 and 468 mg. per 100 ml., respectively.

Xanthelasma of the eyelids was present in 88 (23%) of the 390 persons. No differentiation was made in the "old" series between xanthoma tendinosum and tuberosum; the two forms were present in 25 of the 201 persons. In the "new" series of 189 persons, 19 (10%) had xanthoma tendinosum and only 3 (1.6%) xanthoma tuberosum.

In both the old and new groups, coronary artery disease was present in 168 (43%) of the 390 persons. The high incidence of coronary artery disease resulted in part because most index patients sought treatment for their cardiac disorders. Nevertheless, the findings indicate that the most frequent abnormalities among members of these families were elevated serum cholesterol concentration and coronary atherosclerosis. Xanthelasma of the eyelids and xanthoma tendinosum were next in frequency, whereas xanthoma tuberosum was seen least often.

CLINICAL ASPECTS

A comparison of idiopathic hyperlipemia and idiopathic hypercholesteremia in their clinical and chemical manifestations is given in Tables 1 and 2. Among the index patients with either disorder and among their family members there is a high incidence of coronary artery disease: 34% among those with idiopathic hyperlipemia and 43% among those with idiopathic hypercholesteremia. The difference in incidence of coronary artery disease might be in part caused by differences in selection of the patients in the two groups, as was stated above. While a considerable number of the index patients with idiopathic hy-

TABLE 1.—*Comparison of Two Errors of Lipid Metabolism—Incidence and Clinical Data*

	Idiopathic Hyperlipemia	Idiopathic Hypercholesteremia
Number of index patients with family study	20	77
Number of persons in the families	84	341
Number of individual patients without family study	5	49
Total number of persons	89	390
Males	58	224
Females	31	166
Xanthelasma of the eyelids	2 (2%)	88 (23%)
Xanthoma tendinosum	1 (1%)	19 (10%)*
Xanthoma tuberosum	12 (14%)	3 (2%)*
Coronary artery disease	30 (34%)	168 (43%)
Recurrent pancreatitis	5 (6.6%)	1 (0.25%)
Diabetes	6 (7%)	5 (3%)*
Asymptomatic index patients	4	8

* This number is based on the study of 189 persons of the "new" group.

TABLE 2.—*Comparison of Two Errors of Lipid Metabolism—Chemical Data*

	Idiopathic Hyperlipemia	Idiopathic Hypercholesteremia*
Lactescence of serum	28	0
Lipemia retinalis	3	0
Average serum total cholesterol		
Index patients	623 mg. %	366 mg. %
All persons	412 mg. %	319 mg. %
Average serum esterified cholesterol		
Index patients	377 mg. %	263 mg. %
All persons	260 mg. %	231 mg. %
Average serum phospholipids		
Index patients	588 mg. %	342 mg. %
All persons	420 mg. %	314 mg. %
Average serum neutral fat		
Index patients	2,387 mg. %	436 mg. %
All persons	2,541 mg. %	468 mg. %
Average total serum lipids		
Index patients	3,598 mg. %	1,144 mg. %
All persons	3,373 mg. %	1,101 mg. %

* The chemical data presented in this column are based on the study of 189 persons of the "new" group.

perlipemia came under medical care for noncardiac disorders such as pancreatitis and diabetes, those with idiopathic hypercholesteremia were treated almost exclusively for cardiac disease. The incidence of 33% of coronary artery disease among the families with idiopathic hyperlipemia supports the concept that this error of lipid metabolism represents a predisposition to coronary artery disease not too different from that found in idiopathic hypercholesteremia.‡ The observations justify a revision of the old premise that idiopathic hyperlipemia is simply an innocuous abnormality of the blood.

While some information is at hand concerning the incidence of idiopathic hypercholesteremia in the population at large,§ no corresponding data on idiopathic hyperlipemia are available. Based on a rough estimate, idiopathic hypercholesteremia appears to be 5 to 6 times as frequent as idiopathic hyperlipemia. Both idiopathic hyperlipemia and idiopathic hypercholesteremia were encountered more frequently among men. In idiopathic hypercholesteremia men represented 57% of the total number observed. In idiopathic hyperlipemia the preponderance of men was even more pronounced: 65% were men and 35% women. The sex differences are further illustrated by the figures obtained in the index patients of the two series. Among those with idiopathic hyperlipemia there were 18 men and 7 women (72% male). In the idiopathic hypercholesteremia group there were 74 men and 52 women (59% male).

Of the 25 index patients with idiopathic hyperlipemia, 6 had diabetes mellitus of the mild variety usually associated with moderate or severe obesity. Only two of these patients required insulin; in the others good diabetic control could be achieved by diet regulation alone. In five of these patients there was evidence of vascular disease; four, two of whom had mild hypertension, exhibited evidence of coronary artery disease, and the fifth had thrombosis of the central retinal vein with loss of vision in one eye. The syndrome of idiopathic hyperlipemia, diabetes mellitus, and vascular disease has been described elsewhere,[8] based on observation of these patients over a period of many months to many years. Among 62 index patients of the "new" group with idiopathic hypercholesteremia, 3 had mild diabetes. Based on this small number, it appears that diabetes occurs more frequently in families with idiopathic hyperlipemia (6.7%) than in those with idiopathic hypercholesteremia (2.6%).

Five of the twenty-five index patients with idiopathic hyperlipemia presented evidence of relapsing pancreatitis. The diagnosis was established by clinical observations; chemical analysis of the blood, urine, and pancreatic enzymes; roentgen studies of the gastrointestinal tract, and, in one instance, by operation. Poulsen [12] and Klatskin and Gordon [13] stressed the occurrence of relapsing pancreatitis in patients with idiopathic hyperlipemia. Among other pathogenetic possibilities, that of fat emboli in the pancreatic vessels was considered. In each of the patients with recurrent pancreatitis of this series considerable additional elevation of all serum lipids but especially of neutral fats and total lipids was noted during the attacks and shortly thereafter. One of these patients had diabetes mellitus of the mild variety described above. One may reason that diabetes mellitus in these patients may be secondary to associated pancreatitis in idiopathic hyperlipemia in some instances, even if a definite history of the former is not obtained.

In the past two years we paid increased attention to disorders of the central nervous system encountered among patients and their families with either of the two errors of lipid metabolism. Cerebral vascular accidents occurred in a number of patients in whom myocardial infarction had been observed prior to or after the onset of central nervous system manifestations. In four patients with idiopathic hyperlipemia recurrent transient cerebral manifestations of a somewhat different type were noted. They were of the petit mal variety of epilepsy in two patients and consisted of short periods of disorienta-

‡ References 6 through 8.

§ References 9 through 11.

tion and loss of memory (amnesia) in two others. Whether these episodes were caused by "little strokes" or perhaps by small fat emboli in the cerebral vessels cannot be decided. There was some similarity between the cerebral episodes and the milder attacks of recurrent pancreatitis encountered among patients with idiopathic hyperlipemia. It must be mentioned, however, that none of the four patients with this form of cerebral involvement exhibited relapsing pancreatitis and that, conversely, none of the five patients with recurrent pancreatitis exhibited any cerebral episodes.

Although both abnormalities of lipid metabolism may be associated with xanthomatosis, there were distinct differences in the variety of clinical manifestations. In the index patients and families with idiopathic hyperlipemia, xanthelasma of the eyelids was noted in 2.2%, xanthoma tendinosum in 1.1%, and xanthoma tuberosum in 13.5%. In contrast, in the "new" group of idiopathic hypercholesteremia, xanthelasma of the eyelids was present in 23%, xanthoma tendinosum in 10%, and xanthoma tuberosum in 1.6%. The data indicate that xanthomatosis in idiopathic hyperlipemia was usually of the tuberosum type, while that seen in idiopathic hypercholesteremia was usually of the tendinosum variety. Xanthelasma of the lids was rare in the former and was often seen in the latter. In contrast, xanthoma of the eruptive type was seen in a few instances in the xanthoma tuberosum variety only. These findings are in accord with those of other observers.‖

A number of index patients with idiopathic hyperlipemia and with idiopathic hypercholesteremia and their close blood relatives remained asymptomatic despite marked abnormalities of their serum lipid pattern over periods of many years. Certain similarities between the clinical behavior of idiopathic hypercholesteremia and gout have been mentioned previously.¶ In gout, several members within one family may exhibit elevation of serum uric acid, but only one or two may show clinical manifestations of gout or exhibit uric acid deposition in the tissues (tophi). This analogy may be extended to idiopathic hyperlipemia. For manifest symptoms to appear in either disorder additional mechanisms must be operating, perhaps at the tissue level. The basic metabolic disturbance in idiopathic hyperlipemia as well as in idiopathic hypercholesteremia, like that in gout, acts only as a conditioning factor. Many additional factors involved in the genesis of atherosclerosis have been considered in extensive reviews recently published.# Among them the time factor must be considered. The concentration as well as the duration of the metabolic defect of lipid metabolism may influence the deposition of these substances in the tissues.*

GENETIC ASPECTS

The hereditary nature of idiopathic hypercholesteremia (familial xanthomatosis) has been emphasized repeatedly.† The mode of transmission of this metabolic fault has been debated but not fully established. This has been due in part to the use of the cutaneous lesions instead of the serum cholesterol levels as a characteristic sign of the disorder. A detailed discussion of the problem was presented elsewhere.‡ Evidence is accumulating that idiopathic hypercholesteremia is transmitted as a dominant trait with incomplete penetrance.§

The study of the "old" group of families with idiopathic hypercholesteremia supported the definition that the abnormal homozygote is characterized by hypercholesteremia and xanthoma.[2] The frequent association of xanthomatosis and coronary artery disease in members of these families might lead to the suggestion that perhaps atherosclerosis like xanthoma represents the homozygote abnormal state. Study of additional families of the "new" group illustrated in Figures 2, 3,

‖ References 6, 7, 14, and 15.
¶ References 2 and 16.
# References 17 through 19.
* References 25 and 26.
† References 2, 20, 22, and 24.
‡ References 1 and 2.
§ References 1, 2, 10, and 24.

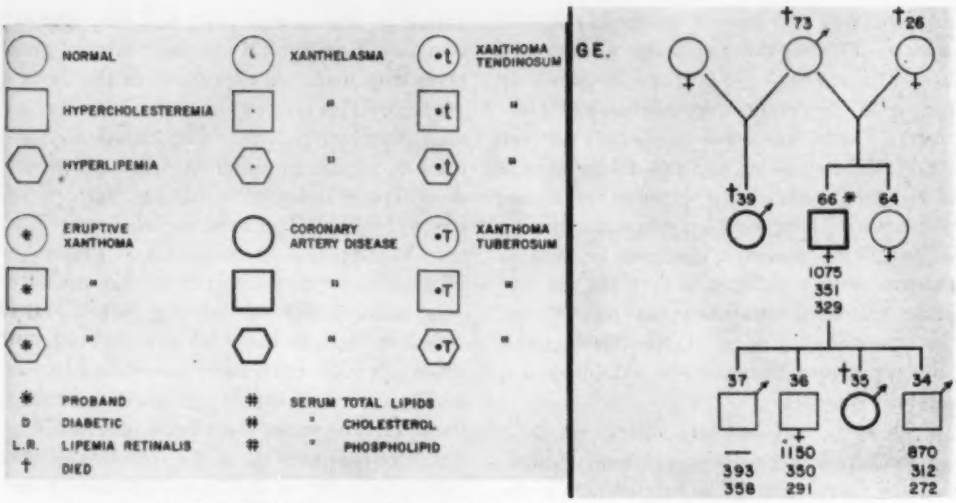


Fig. 2.—The index patient was a 66-year-old woman with coronary artery disease and hypercholesteremia (heterozygote). Mating with a presumed heterozygote (age 64) producing four children, of whom three had elevated serum cholesterol levels and one died of coronary artery disease at age 35. A half-brother died of coronary artery disease at age 39. The numbers above the symbols indicate age. Those below the symbols indicate serum lipids in the following order: total lipids, total cholesterol, phospholipid. For explanation of symbols, see left part of this Figure.

and 4 in which there was a relatively high frequency of coronary artery disease would seem to support such a concept. Such cannot be the case, however, as was stressed previously, since not all patients with coronary artery disease exhibit hypercholesteremia and xanthoma. The study of the "old" and the "new" series of idiopathic hypercholesteremia lends support to the opinion that by definition the abnormal homozygote will have hypercholesteremia and skin or tendon xanthoma.

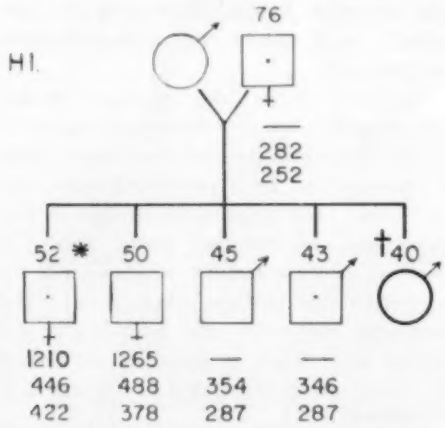


Fig. 3.—Mating of a homozygotic mother (present age 76) with a presumed normal or heterozygotic father producing five children, of whom four had hypercholesteremia and one died of coronary artery disease. For explanation of symbols see Figure 2.

Investigation of families of patients with idiopathic hyperlipemia revealed the interesting fact that among these families both errors of lipid metabolism, i. e., idiopathic hyperlipemia and idiopathic hypercholesteremia, may be seen. Figures 5 and 6 present such "mixed" families.

In the family presented in Figure 5, the index patient was a mild diabetic who showed marked lactescence of the serum and the typical chemical findings of idiopathic hyperlipemia. Three of his sons, all in perfect health, had evidence of idiopathic hypercholesteremia, but no hyperlipemia, and their sera were always clear. A fourth son was known to have diabetes but was not available for study.

Many instances of coronary artery disease occurred in the family presented in Figure 6. The index patient as well as his brother both had idiopathic hyperlipemia. While the index patient had hepatomegaly, his brother was completely asymptomatic. Three other sib-

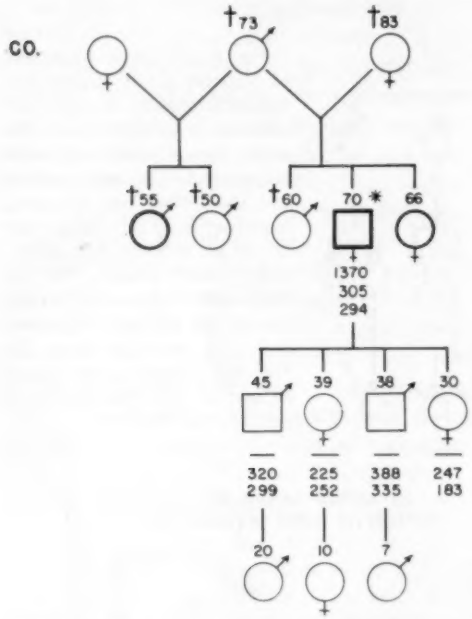


Fig. 4.—Mating of a known heterozygote with a presumed heterozygote producing four children, of whom two were hypercholesteremic. The index patient and her sister had coronary artery disease. One half-brother died of this disease. For explanation of symbols see Figure 2.

lings were healthy and showed a normal serum lipid partition. Their father as well as two of their paternal uncles died of coronary artery disease. A paternal aunt, age 68, had two attacks of myocardial infarction and exhibited idiopathic hypercholesteremia but no hyperlipemia; her serum was always clear in the fasting state. One of her three children had a myocardial infarction at age 42.

Of four siblings, ages 2 to 11, of a Puerto Rican family, two had idiopathic hyperlipemia and hepatosplenomegaly (ages 6 and 2). Their serum showed marked lactescence. The serum of the two other siblings was clear in appearance, although the older of them, age 11, had idiopathic hypercholesteremia, while the other, age 5, exhibited a normal serum lipid partition.

Despite the small number of families with idiopathic hyperlipemia presented in this study, the impression was gained that genetically, at least, both disorders are closely related. One may be considered a variant of the other. Under these conditions idiopathic hyperlipemia and idiopathic hypercholesteremia are perhaps similarly transmitted by a dominant trait with incomplete penetrance.

BIOCHEMICAL ASPECTS

The biochemical differences between idiopathic hyperlipemia and idiopathic hypercholesteremia were reflected in the serum lipid partition estimated by chemical analysis, in the serum lipoproteins determined by paper electrophoresis, in serum lipids and lipoproteins after administration of heparin, and in certain phenomena observed in exogenous and endogenous cholesterol metabolism when studied with various isotopes.

Serum lipid partitions of both groups are compared in Table 2. The lactescence of the serum which characterizes idiopathic hyperlipemia as an entity is associated with profound elevation of neutral fat and total lipid as well as an increase in serum cholesterol and phospholipids far above the levels encountered in idiopathic hypercholesteremia. Only in the mildest instances was there an elevation of neutral fat and total lipids with normal levels of cholesterol and phospholipids. If the defective fat clearance of the serum was controlled by dietary adjustment (low-fat diet), the lipid pattern of patients with idiopathic hyperlipemia closely resem-

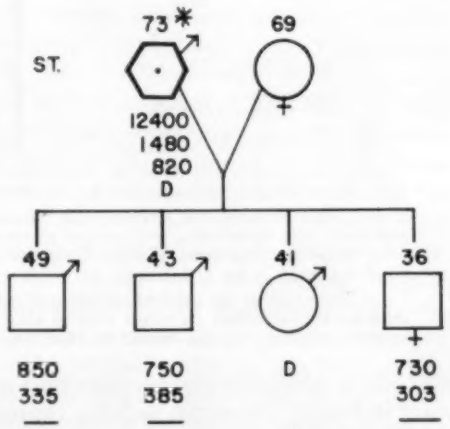


Fig. 5.—Three children of the index patient with idiopathic hyperlipemia exhibited idiopathic hypercholesteremia. For explanation of symbols see Figure 2.

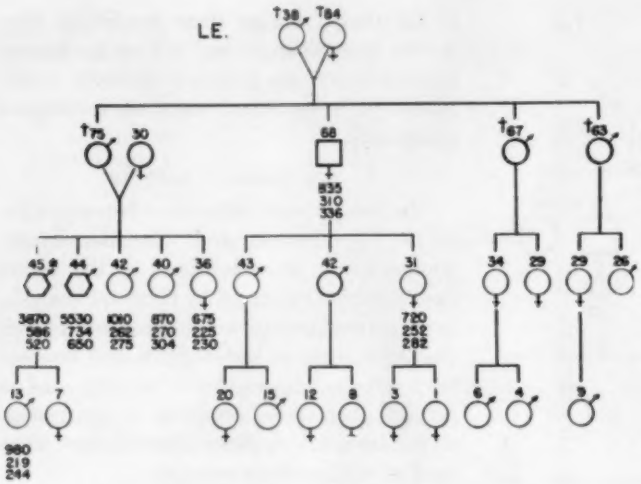


Fig. 6.—Frequent occurrence of coronary artery disease was seen in this family in which both idiopathic hyperlipemia and idiopathic hypercholesteremia were encountered. The index patient and his brother had idiopathic hyperlipemia. Their father and two of his brothers died of coronary artery disease. The father's sister had coronary artery disease and idiopathic hypercholesteremia; one of her three children had coronary artery disease at age 42. For explanation of symbols see Figure 2.

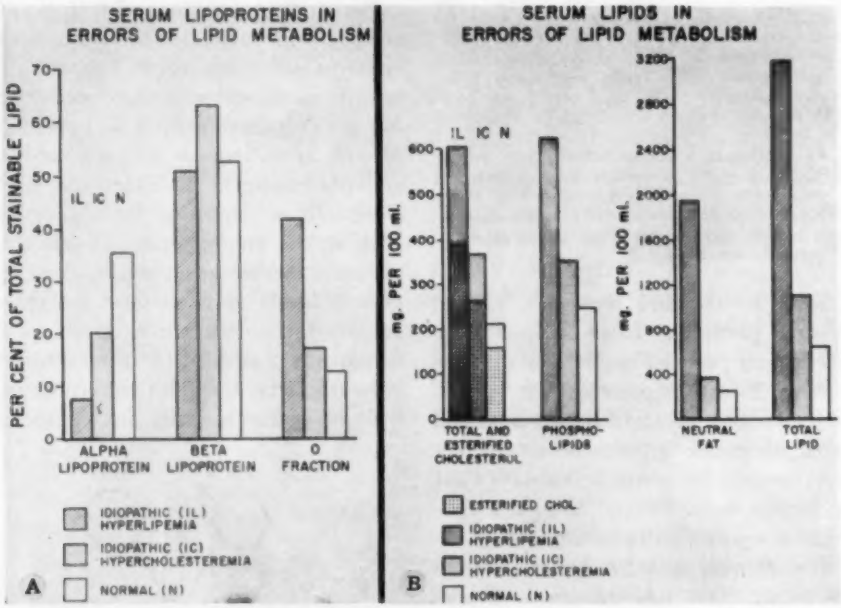


Fig. 7.—Comparison of serum lipoproteins and lipids in 13 patients with idiopathic hyperlipemia and 29 with idiopathic hypercholesteremia with those seen in 10 normal persons.

A, Note in idiopathic hyperlipemia, marked depression of alpha-lipoprotein, no significant change in beta-lipoprotein, and marked elevation of the O-fraction; in idiopathic hypercholesteremia moderate depression of alpha-lipoprotein, moderate elevation of beta-lipoprotein, and only slight increase in the O-fraction.

B, Note further the marked elevation of all serum lipid fractions in idiopathic hyperlipemia in contrast to the milder increases seen in idiopathic hypercholesteremia. The figures encountered in these small groups are similar to those seen in the large series presented in Table 2.

bled that of idiopathic hypercholesteremia in many instances. The serum turbidity cleared up to a great extent, or even completely. There was a precipitous fall in serum neutral fat and total lipid and a concomitant decrease of serum cholesterol and phospholipid to levels often seen in idiopathic hypercholesteremia. In severe instances of idiopathic hyperlipemia, however, the response to low-fat diet was less satisfactory.

As seen previously in patients with idiopathic hyperlipemia and diabetes,[5] even transient transgressions of the low-fat diet resulted promptly in increased lactescence and elevation of all serum lipids lasting for several days or longer. Again, this elevation affected especially serum neutral fat, and to a less extent cholesterol and phospholipids. The rise and fall of serum cholesterol and phospholipids concomitant with the increase or decrease of neutral fat suggest that the metabolic defect of idiopathic hyperlipemia involves more than inability to clear the serum of neutral fat and supports the premise that this disorder of lipid metabolism is, in addition, an exaggerated form of idiopathic hypercholesteremia.[20] It may be recalled that genetic studies of the families led to the conception that one disorder may represent a variant of the other.

Studies of serum lipoproteins were performed in 14 patients with idiopathic hyperlipemia, 11 men and 3 women, average age 47 years. Compared with a series of normal men and women, patients in this group exhibited marked depression of alpha-lipoprotein and marked elevation of the O-fraction (lipid adsorbed at the point of application of the serum and representing mainly neutral fat), while beta-lipoprotein concentration was unaltered. Similar studies in 29 patients with idiopathic hypercholesteremia (14 women and 15 men), average age 47 years, revealed only moderate depression of alpha-lipoprotein, moderate elevation of beta-lipoprotein, and slight increase in the O-fraction. However, in all instances visual study of the patterns revealed the beta-lipoprotein peak to be in the form of a sharp spike which appeared characteristic of idiopathic hypercholesteremia. The data obtained in both groups are summarized in Table 3, and Figure 7. They shed additional light on the metabolic differences that exist between the two errors of lipid metabolism.[27] Important results obtained with other techniques have been previously reported by other authors.‖

Studies of serum lipoproteins of patients with idiopathic hyperlipemia following intravenous administration of heparin revealed marked alteration of the profile. The O-fraction and beta-lipoprotein spread out and merged with an increasingly mobile alpha component. Similar changes were not seen in patients with idiopathic hypercholesteremia. The effect of heparin on serum lipid partition in disturbances of lipid metabolism was previously reported.[36]

TABLE 3.—*Lipoprotein Profile and Lipid Partition of the Serum in Patients with Idiopathic Hyperlipemia and Idiopathic Hypercholesteremia*

			Lipoproteins, % of Total Stainable Lipid				Lipids, Mg./100 Ml.			
Group	No.	Age	Alpha	Beta	O-Fraction	Beta+O	Cholesterol/ Esters	Phospholipids	Neutral Fat	Total Lipid
Idiopathic hyperlipemia	13	47	7.5±5.1	50.7±8.1	41.7±8.9	92.4±5.1	605/398 ±222	±123 624±206	1935±394	3164±1620
Idiopathic hypercholesteremia	29	48	20.0±7.8	63.0±6.8	17.0±6.5	80.0±7.8	370/202 ±79	±61 351±49	355±124	1079±187
Normal	10	37	35.3±6.7	52.4±7.2	12.3±3.8	64.7±6.7	211/157 ±31	±25 242±38	176	629

The isotope studies in both errors of lipid metabolism were performed by Hellman, Rosenfeld, and Gallagher in collaboration with our group.[37] They dealt with the behavior of cholesterol derived as such from the diet in comparison with cholesterol synthesized endogenously from acetate. Acetate and cholesterol were labeled with either radioactive carbon or radioactive hydrogen (H_3 or tritium). The studies of endogenous and exogenous cholesterol metabolism, therefore, could be carried out separately or simultaneously in the same patient, using ace-

‖ References 28 through 35.

tate labeled with one isotope and cholesterol labeled with the other.

The findings in idiopathic hyperlipemia differed in two respects from those obtained in subjects with normal cholesterol levels and with idiopathic hypercholesteremia.[37] The activity of free serum cholesterol synthesized from acetate declined somewhat more slowly during the first few days than in the other two groups, so that the rising specific curve of ester cholesterol did not intersect the curve of the free cholesterol until about the sixth day. The point of intersection occurred in the other two groups at two to three days. The second difference was noted in the early portion of the specific activity of cholesterol arising from dietary cholesterol. The ester cholesterol specific activities were initially at a higher level, rose more rapidly, and exceeded those of the free cholesterol throughout the experiment. The two curves, therefore, did not intersect in contrast to findings obtained in normocholesteremic persons and in those with idiopathic hypercholesteremia. The latter parts of the curves show a parallel rate of decline of cholesterol radioactivity. If the body distinguishes between endogenously and exogenously derived cholesterol, this could seemingly only occur during the immediate period after absorption or during synthesis.

Our knowledge concerning genetic control of metabolic processes, although advanced by recent experimental work,[38] is very limited. The nature of the inborn errors of lipid metabolism resulting eventually in idiopathic hyperlipemia or idiopathic hypercholesteremia and in predisposition to atherosclerosis is even more obscure. One might suspect that in these abnormal metabolic states a disturbance occurs in genetically controlled enzymatic chain reactions resulting in accumulation of lipid subtances in the blood and eventually in deposition in the tissues. At the present time the knowledge of the enzymatic systems directing synthesis and degradation of cholesterol and other lipids is scant.[38]

SUMMARY

The study comprised 20 families and 5 additional persons with idiopathic hyperlipemia (total 89 persons) and 77 families and 49 additional persons with idiopathic hypercholesteremia (total 390 persons).

Lactescence of serum in the fasting state was noted in all index patients with idiopathic hyperlipemia and in three of their close relatives. Serum total lipids averaged for the group 3373, serum cholesterol 412, esterified cholesterol 260, phospholipid 420 mg. per 100 ml. Xanthelasma of the eyelids was present in 2 persons, xanthoma tendinosum in 1, whereas xanthoma tuberosum was found in 12. Coronary artery disease was present in 30 (34%) of the 89 persons. Of the 25 index patients, 6 had mild diabetes, 5 had relapsing pancreatitis, and 4 were asymptomatic.

The serum of the index patients with idiopathic hypercholesteremia and that of their close blood relatives was clear when examined in the fasting state. For the "new" group of 169 persons, serum total lipids averaged 1101, cholesterol 319, esterified cholesterol 231, and phospholipid 314 mg. per 100 ml. Of the "new" group of 169 persons, 19 had xanthoma tendinosum and only 3 xanthoma tuberosum. Xanthelasma of the eyelids was present in 88 of the entire series of 390 persons. Coronary artery disease was present in 168 (43%) of the entire series of 390. Among the 169 persons comprising the "new" group, 5 had diabetes and 1 relapsing pancreatitis; 8 index patients were asymptomatic.

Both errors of lipid metabolism were encountered more frequently among men than women. The preponderance of men was more pronounced in idiopathic hyperlipemia (65%) than in idiopathic hypercholesteremia (57%).

The data indicated that idiopathic hyperlipemia represented a predisposition to coronary artery disease not too different from that found in idiopathic hypercholesteremia. Xanthomatosis in idiopathic hyperlipemia was usually of the tuberosum type, while that seen in idiopathic hypercholesteremia was of the tendinosum variety.

The syndrome of idiopathic hyperlipemia and mild diabetes mellitus was observed among 6 of the 25 index patients. Five of these had evidence of vascular disease. Five index patients exhibited relapsing pancreatitis, one of whom had diabetes. Diabetes mellitus in idiopathic hyperlipemia might have been secondary to associated pancreatitis even if a definite history of the latter was not obtained.

Cerebral vascular accidents occurred in a number of patients with either of the two errors of lipid metabolism. Four patients with idiopathic hyperlipemia exhibited recurrent transient cerebral manifestations such as petit mal variety of epilepsy, disorientation, and amnesia.

Among close blood relatives of patients with idiopathic hyperlipemia, this error of lipid metabolism as well as that of idiopathic hypercholesteremia was seen. The study of the "mixed" families suggested that both disorders may be closely related and that one may represent a variant of the other.

The biochemical differences between the two errors of lipid metabolism included serum lipid partition (chemical analysis), serum lipoprotein patterns (paper electrophoresis), alteration of both serum lipids and lipoproteins by administration of heparin, and certain phenomena observed in exogenous and endogenous cholesterol metabolism when studied with isotopes.

136 E. 64th St., New York 21.

REFERENCES

1. Adlersberg, D.: Hypercholesteremia with Predisposition to Atherosclerosis: An Inborn Error of Lipid Metabolism, Am. J. Med. **11**:600, 1951.

2. Adlersberg, D.; Parets, A. D., and Boas, E. P.: Genetics of Atherosclerosis: Studies of Families with Xanthoma and Unselected Patients with Coronary Artery Disease Under the Age of 50 Years, J. A. M. A. **141**:246, 1949.

3. Fagge, C. H.: Xanthomatous Diseases of the Skin: I. General Xanthelasma or Vitiligoidea, Tr. Path. Soc. London **24**:242, 1873.

4. Montgomery, H.: Cutaneous Manifestations of Diseases of Lipoid Metabolism, M. Clin. North America **24**:1249, 1940.

5. Bloom, D.; Kaufman, S. R., and Stevens, R. A.: Hereditary Xanthomatosis, Arch. Dermat. & Syph. **45**:1, 1942.

6. Lever, W. F.; Smith, P. A. J., and Hurley, N. A.: Idiopathic Hyperlipemic and Primary Hypercholesteremic Xanthomatosis: I. Clinical Data and Analysis of the Plasma Lipids, J. Invest. Dermat. **22**:33, 1954; II. Analysis of the Plasma Proteins and Lipids by Means of Electrophoresis and Fractionation of the Plasma Proteins; Effect of High Speed Centrifugation and of Extraction with Ether on the Plasma Proteins and Lipids, ibid. **22**:53, 1954; III. Effects of Intravenously Administered Heparin on the Plasma Proteins and Lipids, ibid. **22**:71, 1954.

7. Soffer, A., and Murray, M.: Relationship of Essential Hyperlipemia to Premature Atherosclerosis, Circulation **10**:611, 1954.

8. Adlersberg, D., and Wang, C.: Syndrome of Idiopathic Hyperlipemia, Mild Diabetes Mellitus, and Severe Vascular Damage, Diabetes **4**:210, 1955.

9. Adlersberg, D.; Schaefer, L. E., and Drachman, S. R.: Incidence of Hereditary Hypercholesteremia, J. Lab. & Clin. Med. **39**:237, 1952.

10. Schaefer, L. E.; Drachman, S. R.; Steinberg, A. G., and Adlersberg, D.: Genetic Studies on Hypercholesteremia: Frequency in a Hospital Population and in Families of Hypercholesteremic Index Patients, Am. Heart J. **46**:99, 1953.

11. Adlersberg, D.; Schaefer, L. E.; Steinberg, A. G., and Wang, C.: Genetic Aspects of Idiopathic Hypercholesteremia: Studies of Parents and Offspring in 200 Randomly Selected Families, Circulation **10**:600, 1954.

12. Poulsen, H. M.: Familial Lipemia: New Form of Lipoidosis Showing Increase in Neutral Fats Combined with Attacks of Acute Pancreatitis, Acta med. scandinav. **138**:413, 1950.

13. Klatskin, G., and Gordon, M.: Relationship Between Relapsing Pancreatitis and Essential Hyperlipemia, Am. J. Med. **12**:3, 1952.

14. Lever, W. F.; Herbst, F. S. M., and Hurley, N. A.: Idiopathic Hyperlipemia and Primary Hypercholesteremic Xanthomatosis: IV. Effects of Prolonged Administration of Heparin on Serum Lipids in Idiopathic Hyperlipemia, A. M. A. Arch. Dermat. **71**:150, 1955.

15. Lever, W. F.; Herbst, F. S. M., and Lyons, M. E.: Idiopathic Hyperlipemia and Primary Hypercholesteremic Xanthomatosis: V. Analysis of Serum Lipoproteins by Means of Ultracentrifuge Before and After Administration of Heparin, A. M. A. Arch. Dermat. **71**:158, 1955.

16. Adlersberg, D.: Newer Advances in Gout, Bull. New York Acad. Med. **25**:651, 1949.

17. Gubner, R., and Ungerleider, H. E.: Arteriosclerosis: A Statement of the Problem, Am. J. Med. **6**:60, 1949.

18. Katz, L. N., and Stamler, J.: Experimental Atherosclerosis, Springfield, Ill., Charles C Thomas, Publisher, 1953.

19. Robbins, S. R., and Dack, S.: Atherosclerosis: A Review of Predisposing Factors and the Problems of Treatment, J. Mt. Sinai Hosp. **22**:34, 1955.

20. Thannhauser, S. J.: Lipidoses: Diseases of the Cellular Lipid Metabolism, edited by H. A. Christian, Ed. 2, New York, Oxford University Press, 1950.

21. Thannhauser, S. J., and Schmidt, G.: Lipins and Lipidoses, Physiol. Rev. **26**:275, 1946.

22. Müller, C.: Xanthomata, Hypercholesterolemia, Angina Pectoris, Acta med. scandinav. Supp. 89, p. 75, 1938.

23. Svendsen, M.: Are Supernormal Cholesterol-Values in Serum Caused by a Dominantly Inherited Factor? Acta med. scandinav. **104**:235, 1940.

24. Wilkinson, C. F., Jr.; Hand, E. A., and Fliegelman, M. T.: Essential Familial Hypercholesterolemia, Ann. Int. Med. **29**:671, 1948.

25. Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis: A Survey of the Problem, New York, The Macmillan Company, 1933, p. 309.

26. Wang, C.; Schaefer, L. E., and Adlersberg, D.: Tissue Permeability: A Factor in Atherogenesis, Circulation Res. **3**:293, 1955.

27. Adlersberg, D.; Bossak, E. T.; Sher, I. H., and Sobotka, H.: Electrophoresis and Monomolecular Layer Studies with Serum Lipoproteins, Clin. Chem. **1**:18, 1955.

28. Gofman, J. W., and others: Blood Lipids and Human Atherosclerosis, Circulation **2**:161, 1950.

29. Jones, H. B., and others: Lipoproteins in Atherosclerosis, Am. J. Med. **11**:358, 1951.

30. Keys, A.: Cholesterol, "Giant Molecules," and Atherosclerosis, J. A. M. A. **147**:1514, 1951.

31. Keys, A.: "Giant Molecules" and Cholesterol in Relation to Atherosclerosis, Bull. Johns Hopkins Hosp. **88**:473, 1951.

32. Review: Atherosclerosis, Serum Cholesterol and Lipoproteins, Nutrition Rev. **9**:167, 1951.

33. Gould, R. G.: Lipid Metabolism and Atherosclerosis, Am. J. Med. **11**:209, 1951.

34. Barr, D. P.: Some Chemical Factors in the Pathogenesis of Atherosclerosis, Circulation **8**:641, 1953.

35. Page, I. H.: Atherosclerosis: An Introduction, Circulation **10**:1, 1954.

36. Herzstein, J.; Wang, C., and Adlersberg, D.: Effect of Heparin on Plasma Lipid Partition in Man: Studies in Normal Persons and in Patients with Coronary Atherosclerosis, Nephrosis and Primary Hyperlipemia, Ann. Int. Med. **40**:290, 1954.

37. Hellman, L.; Rosenfeld, R. S.; Eidinoff, M. L.: Fukushima, D. K.; Gallagher, T. F.; Wang, C., and Adlersberg, D.: Isotopic Studies of Plasma Cholesterol of Endogenous and Exogenous Origins, J. Clin. Invest. **34**:48, 1955.

38. Beadle, G. W.: Genetic Control of Biochemical Reactions, Harvey Lect. (1944-1945) **40**:179, 1945.

Hemangioendothelioma of Liver Following Thorium Dioxide Administration

HENRY TESLUK, M.D.
and
WARREN A. NORDIN, M.D., Detroit

The use of thorium dioxide (Thorotrast) as a radiopaque material in the diagnosis of vascular aberrations, especially in the case of liver disease, had at one time widespread popularity. There has been considerable discussion of toxic effects based upon animal experimentation, but actually little information is available concerning its late effects in man. Recently we have had the opportunity of observing unusual lesions in the liver of a person who had been given thorium dioxide intravenously several years previously. The purpose of this paper is to present these findings together with a discussion of the relationship of the lesions to thorium dioxide administration.

REPORT OF A CASE

The patient was a 54-year-old white machinist, who entered Henry Ford Hospital because of an illness characterized by jaundice and abdominal pain. Six months previously he noted loss of energy, and one month previously he developed anorexia together with bouts of constipation and diarrhea. Three weeks earlier, cramping intermittent epigastric pain was noted. He began to vomit one week prior to admission and jaundice became noticeable. There was no melena, hematemesis, or previous jaundice. Some weight loss had occurred.

His past history was not unusual except for moderate consumption of alcohol. Fourteen years previously he had what was thought to be a cerebrovascular accident, and during the investigation he was given thorium dioxide intra-arterially for cerebral angiography.

On examination he was cachectic and jaundiced. Rectal temperature was 100.8 F; pulse rate, 110 per minute, and respiration rate, 16 per minute. The abdomen was distended and a fluid wave was present. The liver was tender and enlarged to 5 cm. below the costal margin. The spleen was not palpable. Otherwise no unusual features were noted. Laboratory studies gave the following data: hemoglobin, 9.7 gm. per 100 cc.; erythrocytes, 3,700,000 per cubic millimeter; leucocytes, 25,050 per cubic millimeter with 94% neutrophiles and 6% lymphocytes; smear, anisocytosis, poikilocytosis, and hypochromia; CO_2 combining-power, 13.5 volumes per cent; chloride, 97 mg. per 100 cc.; sodium, 132 mg. per 100 cc.; prothrombin time, 32 seconds (23%); thymol flocculation, negative; thymol turbidity, 1 unit; cephalin-cholesterol flocculation, 3+; bilirubin: total 6.8 mg. per 100 cc., direct 2.72 mg. per 100 cc.; calcium, 9.9 mg. per 100 cc.; serum amylase, 60%. X-rays showed no free air in the abdomen, but both the liver and spleen were visualized as slightly radiopaque masses with tiny flecks also seen in the spleen.

A paracentesis was done, and 3500 cc. of bloody fluid was obtained from the abdomen. The patient's blood pressure fell to 88/40 mm. Hg, and 1500 cc. of blood was required to raise the pressure to normal.

On the following day an abdominal operation was done, and 2000 cc. of bloody fluid was aspirated from the abdomen during this procedure. A cholecystostomy was carried out, as well as a biopsy of the liver. The patient's blood pressure was low postoperatively despite proper therapy, and marked bloody drainage ensued from the incision. He died on the evening of the same day.

Autopsy Findings.—The body was that of an adult elderly white man showing evidence of some weight loss and moderate jaundice. There was oozing of bloody fluid from the abdominal incisions, but the abdomen was not distended. Subcutaneous ecchymosis was noted in the right flank. When opened, the abdomen contained about 1000 cc. of bloody fluid, and considerable ecchymosis was encountered in the subperitoneal tissues. The liver

Submitted for publication July 15, 1955.

Department of Pathology, Henry Ford Hospital.

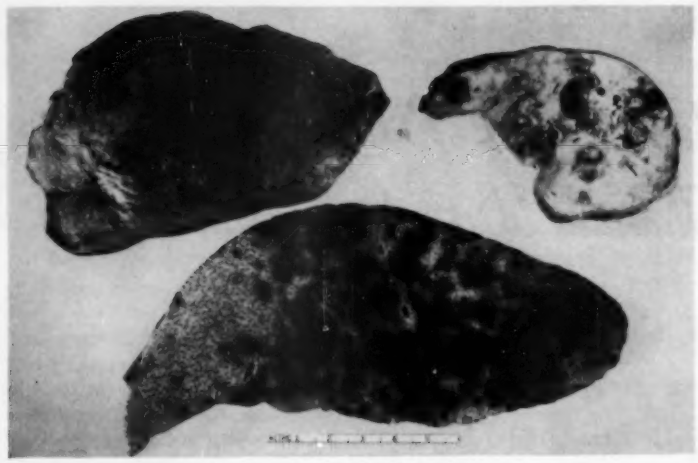


Fig. 1.—Liver, upper left and lower; spleen, upper right. Numerous hemorrhagic angiomatous lesions can be seen through the intact capsule of the liver and are clearly demonstrated in the sectioned surface. Spleen is fibrotic and shows, in addition, a large area of infarction.

was greatly enlarged, extending 15 cm. below the costal margin. It weighed 5000 gm. and presented an unusual appearance (Fig. 1). The capsule was smooth, but within the parenchyma were a large number of hemorrhagic cystic spaces ranging in size from 2 to 3 cm. to barely visible lesions. The cut surface showed a honeycombing by these lesions which were more numerous in the superior portion of both major lobes, where actual coalescence occurred. Where less involved hepatic tissue was available, it showed evidence of fatty replacement together with central lobular necrosis. The extrahepatic biliary system was normal except for the presence of the cholecystostomy.

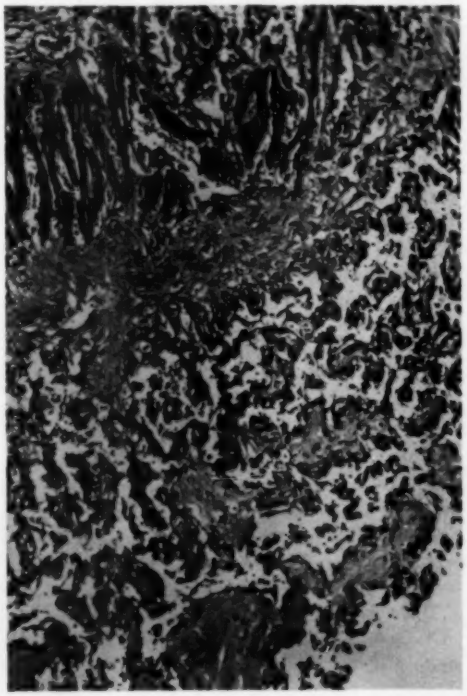
The spleen was also unusual (Fig. 1). It weighed 260 gm. and was pink-gray in color. The capsule was thickened, and the organ was firm. On the lateral aspect an organizing 3×4 cm. infarct was present. The cut surface of the spleen was smooth and pale with no evidence of follicular structures.

At the gastroesophageal junction there was ulceration of the mucosa together with distention of the submucosal veins. A few areas of submucosal ecchymoses were encountered in the large bowel. The heart weighed 400 gm., and a 3 cm. lipoma was attached to the left auricular epicardium. Both lungs showed moderately prominent edema.

MICROSCOPIC EXAMINATION

Liver.—The peculiar lesions seen grossly were found to consist of focal angiomatous structures which varied considerably in size from large cyst-like areas to microscopic nodules. In some areas they were practically confluent and in others, sparsely scattered about. When small enough to permit orientation, they seemed to arise most frequently adjacent to portal areas. The largest ones showed central cyst formation surrounded by curious fibrous trabeculae resembling,

Fig. 2.—Wall of large endotheliomatous cyst in liver. Trabecular arrangement of fibrous tissue surrounded by endothelial cells is apparent. Adjacent liver tissue in the upper portion of the picture shows compression and atrophy. Reduced about ⅔ from mag. × 140.



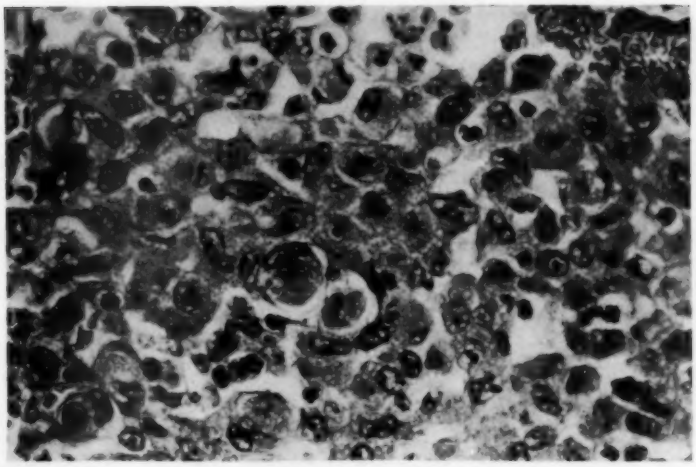


Fig. 3—Syncytial mass composed entirely of proliferating endothelial cells from one of the hepatic lesions. The large nucleus and prominent nucleolus are clearly shown. Reduced about ⅔ from mag. × 700.

somewhat, villiform placental structures and merged peripherally with more compact fibrous tissue (Fig. 2). These cystic spaces and the sinusoidal channels between the trabeculae were lined by proliferating endothelial cells, sometimes in a single layer and sometimes in a thick membrane. They frequently formed syncytial and papillary masses (Fig. 3). The spaces were sometimes empty but usually contained blood. When the lesions were small, the trabeculae were composed of hepatic cells either in good preservation or undergoing partial replacement by connective tissue, and the sinusoidal channels merged gradually with normal intralobular sinusoids (Fig. 4). In the microscopic foci there was only a mild dilation of normal sinusoids together with focal nodules of proliferating endothelial cells. The hyperplastic endothelial cells closely resembled normal Kupffer cells, except that they were somewhat larger with large nuclei. The chromatin pattern was delicate with occasional larger fragments, and the nucleoli were unusually prominent. Cytoplasm was clear or faintly granular and was only moderate in quantity. Cellular outlines were poorly demarcated, and frequently syncytial masses were present. Mitotic figures were rare. There was evidence of phagocytosis, fragments of erythrocytes and hemosiderin being involved. There was no evidence of necrosis

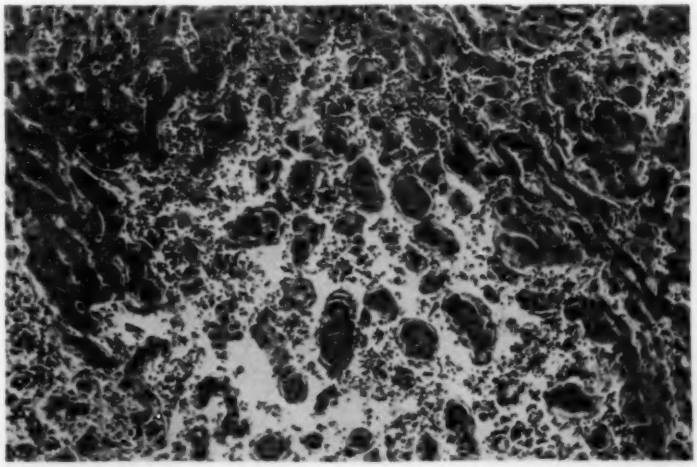


Fig. 4.—Medium-sized cyst from liver. Preservation of hepatic cells in the cords around the periphery of the lesion is still good. Investment by proliferating endothelial cells is also demonstrated. Reduced about ⅔ from mag. × 140.

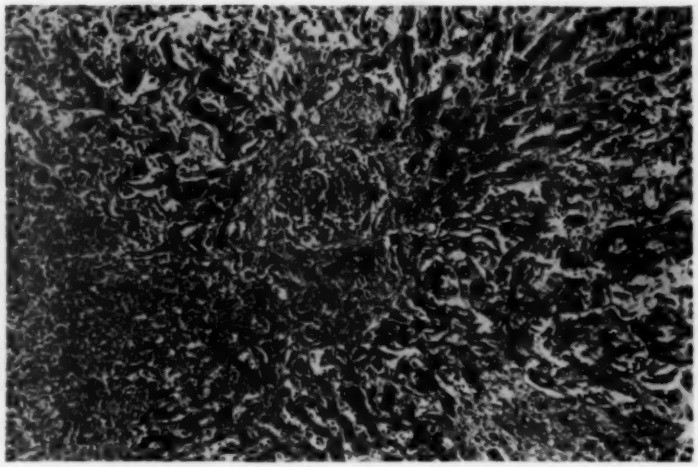


Fig. 5.—Portal area from liver showing scattered masses of thorium dioxide in macrophages. There is mild fibrosis. Reduced about ⅜ from mag. × 140.

or degeneration of these cells. Occasionally it appeared as though small radicles of the portal system contained masses of the proliferating endothelial cells, and in some portal areas there was a suggestion of invasion into supporting connective tissue.

In other areas where hepatic tissue was less involved by the presence of these endotheliomatous lesions, the lobules showed acute hemorrhagic central necrosis which was thought to be subsequent to anoxia. The portal areas showed a moderately prominent acute and chronic inflammation together with occasional inspissated bile plugs in biliary radicles. In many Kupffer cells, especially in areas of fibrosis and in portal areas, a light-brown amorphous globular material was seen (Fig. 5). In other areas small deposits were seen lying free in fibrous tissue. The globules varied in size and were refractile but nonpolarizing. These were negative when stained for iron. Although they were roughly related to areas of fibrosis, the relation was not invariable.

Spleen.—There was marked fibrosis with almost complete depletion of lymphoid tissue. Sinusoidal channels persisted between the fibrotic septae, and these were lined by prominent reticuloendothelial cells (Fig. 6). Large masses of the pigment encountered in the liver were present in fibrous areas and around larger arteries (Fig. 7). There was

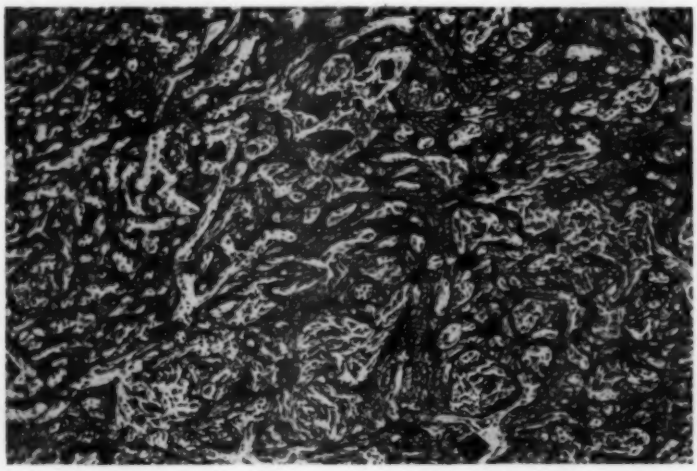


Fig. 6—Spleen. Reticulum stain. Proliferating endothelial cells are clearly visible within the reticulum pattern and constitute a fair proportion of the tissue present. Reduced about ⅜ from mag. × 140.

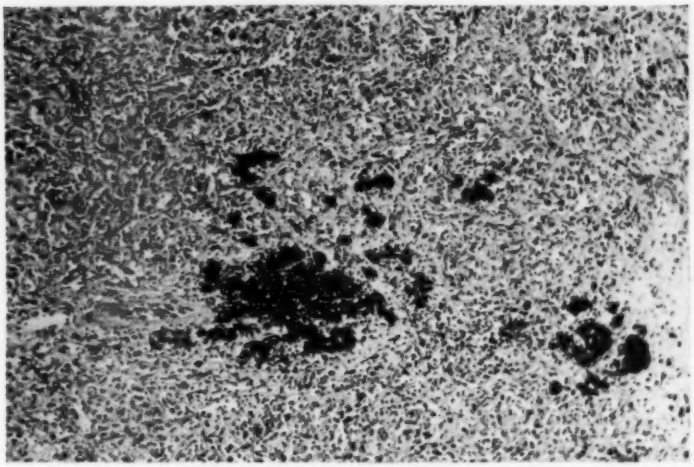


Fig. 7.—Spleen showing large masses of thorium dioxide. Material was more abundant in the spleen. Note the extensive fibrosis together with absence of lymphoid tissue. Reduced about ⅓ from mag. × 140.

much more pigmentary deposit encountered in the spleen when compared with that in the liver. The usual changes of necrosis and organization were found in the infarcted area. Occasional recently thrombosed vessels were also noted. Lymph nodes showed marked edema and evidence of acute and chronic lymphadenitis, but no pigmentary deposits were found. There was increased cellularity in the bone marrow, and numerous macrophages containing the amorphous pigment granules were present. Nowhere was there any evidence to suggest that the endotheliomatous lesions in the liver had involved other organs.

The granular pigment encountered in the tissue was examined in greater detail by various techniques. When blocks of liver or spleen were placed in a Geiger counter, there was evidence of radioactivity but no quantitative measurements were made. Radioautographs were also done, and deposits of the pigment produced positive changes in the emulsion employed. Another method was also used which had been applied to thorium dioxide once before by Birkner,[1] and which may be useful in future samples. This was done by the x-ray diffraction technique. This was carried out with the cooperation of Jonathan Parsons, physicist at the Edsel Ford Institute for Research. Figure 8 shows that thorium dioxide has a characteristic, easily determined pattern. A random block of splenic tissue was homogenized in distilled water and the sediment after drying was inserted into a capillary tube. When this was subjected to x-ray diffraction studies, a pattern was obtained displaying the same diffraction lines (Fig. 8).

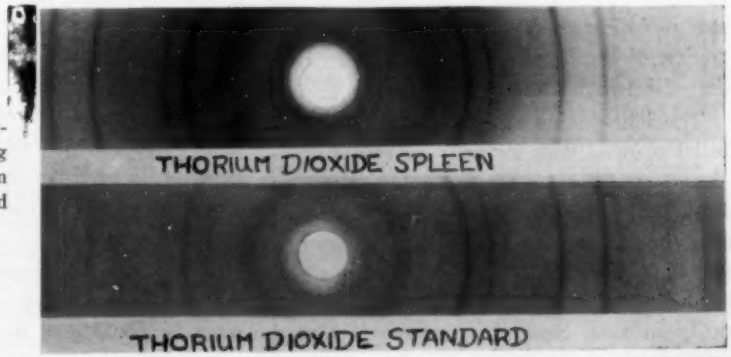


Fig. 8.—X-ray diffraction patterns showing marked similarity between control material and actual tissue analysis.

COMMENT

The material called Thorotrast is a colloidal suspension of thorium dioxide, and according to a report of the Council on Pharmacy and Chemistry of the American Medical Association (1932)[2] it contains about 25% of ThO_2 by volume and 19% to 20% by weight. Thorium dioxide is actually a mixture of isotopes, their proportions depending on the degree of purification and the time lapsed since purification. Within 14 years after purification nearly 75% of the equilibrium amount of $_{89}Ac^{228}$ (mesothorium II) is restored in the mixture, and this gradually decomposes into $_{90}Th^{228}$ (radiothorium), the most dangerous of the isotopes (Baillif).[3] According to Schlund,[4] the radioactivity is primarily in the form of alpha rays, 25 ml. of thorium dioxide being equivalent to a maximum of 1γ and a minimum of 0.5γ of radium. The beta and gamma ray emission is too weak to be of physiological significance according to this worker. Since a total of 75 ml. of thorium dioxide is ordinarily employed in intravenous studies, the equivalent in alpha ray activity of from 1.5γ to 3.0γ of radium is distributed, with primary localization in liver and spleen. Elimination studies have not been complete or thorough, and claims vary from a 50% diminution in three months to no elimination in eight months. The material had been employed on a wide scale previously, being introduced by Blühbaum and co-workers[5] in 1928 in roentgen visualization. It was used for a while in gastroenterology, then in urology, and later in neurology. It was also applied to the study of diseases of the liver, since concentration of thorium in liver and spleen renders them radiopaque.

The long-lasting radioactivity of the material coupled with its poor excretion occasioned considerable alarm as to its use in man. Numerous studies have been employed in animals, such as the report of Roussy, Oberling, and Guérin,[6] who made a series of injections intraperitoneally or subcutaneously in rats. Several sarcomas developed which could be serially transplanted, but no metastases were noted. Several papers have also appeared recording the local effect of thorium dioxide in man, usually in cases of extravasation. These so-called tumors appear to represent foreign-body granulomas rather than true neoplasms. An extensive study of long-term results in man was published by Yater and Coe.[7] They had a 10-year follow-up on 286 patients in whom thorium dioxide hepatosplenography had been carried out. Of these, 189 had died at varying times after administration, most of them within a year. Only four had survived more than seven years. Two of these cases at autopsy showed diffuse liver disease, one being considered a Laennec's cirrhosis, the other a syphilitic cirrhosis. There were 67 patients lost to follow-up and 30 others who were still alive. These were in good health. The authors even believed that the patients with preexisting liver disease in this group had done better than normally expected. Although there was some diminution in the amount of material in the liver and spleen, it could still be visualized by x-ray in these patients. They believed that there was no immediate or remote ill effect of importance in this group. Jacobson and Rosenbaum[8] also published a study of postmortem findings five years after injection of thorium dioxide and encountered only mild fibrosis of liver and spleen. Large amounts of thorium were seen in these tissues. Cassel and co-workers[9] described studies in three patients, 13, 16, and 17 years after thorium dioxide administration. Portal cirrhosis was encountered in two, but they believed that there was no other evidence of deleterious effect. Cases of severe depression of hematopoiesis have also been reported as following thorium dioxide administration. Moeschlin and co-workers[10] described a fatal panmyelopathy seven years after intra-arterial use of thorium dioxide for an arteriogram.

It had been suggested by earlier writers that the danger of neoplastic transformation must be considered in the use of an agent

Data on Thorium Dioxide Administration with Respect to Late Effects in Man

Year	Author	Purpose	Latent Period, Yr.	Tumor
1947	MacMahon, Murphy, and Bates [11]	Hepatosplenography	12	Endothelial sarcoma, liver
1948	Rudolphi [13]	Dacryocystography	35	Basal-cell carcinoma
1949	Zollinger [14]	Pyelography	16	Spindle-cell sarcoma, kidney
1950	Abrahamson, O'Connor, and Abrahamson [17]	Hepatosplenography	16	Multicentric alveolar cell carcinoma, lung
1951	Horta [16]	Cerebral angiography	3	Endothelial sarcoma, liver
1952	Vögtlin and Minder [15]	Bronchography	18	Squamous-cell carcinoma of the bronchus
1953	Lüdin [10]	Arteriography	14	Hemangioendothelioma of liver and spleen
1954	Heitmann [16]	Hepatosplenography	20	Carcinoma of bile duct and liver

with the radioactive properties of thorium dioxide. However, many years passed before such a case was encountered. The first example was reported by MacMahon and co-workers [11] and consisted of what they regarded as an endothelial sarcoma of the liver arising in a patient 12 years after receiving 75 ml. of thorium dioxide intravenously for hepatosplenography. The patient died as a result of intra-abdominal hemorrhage from cystic hemorrhagic lesions in the liver. These were composed of foci of actively proliferating endothelial cells and were thought by the authors to represent a hemorrhagic endothelial sarcoma with intrahepatic spread. They based their diagnosis on cellular characteristics plus intravascular location of cell masses. However, mitotic figures were extremely uncommon, and the lesion was restricted to the liver. The unusual character of the neoplasm suggests strongly that this was a bona fide example of a change produced by thorium dioxide. Some authors have, however, cast doubt upon the report because of the fact that the patient had received therapy for preexisting syphilis of the liver. Nevertheless, a similar lesion has been produced experimentally in a rabbit by Zeitlhofer and Speiser.[12] The animal died four years after intravenous injection with thorium dioxide. Autopsy showed multiple hemorrhagic lesions of the liver, spleen, and lungs, and death had occurred owing to the rupture of one of these in the spleen. The lesions were hemangioendotheliomas with marked proliferation of endothelial cells and were especially prominent in the liver.

After MacMahon's report in 1947, a few other cases have been described in which neoplasms were encountered and thought to be related to previous administration of thorium dioxide (Table). Three of these are unusual in that other routes of administration were employed and the tumors elicited in the organ principally involved. Rudolphi [13] described an epidermoid carcinoma occurring at the site of thorium dioxide visualization of a lachrimal duct 35 years previously. A spindle-cell sarcoma of the kidney following pyelography 16 years previously was reported by Zollinger.[14] The third case was that of a squamous-cell carcinoma of the lung in a patient who had a bronchography using thorium dioxide. This case, reported by Vögtlin and Minder,[15] also showed numerous granulomatous lesions in the lung in which thorium dioxide particles were demonstrated. There have been a few other patients who have developed neoplasms after intravascular use of the material. Heitmann [16] reported a carcinoma of the common bile duct developing 20 years after intravenous injection of thorium dioxide in the diagnosis of an Echinococcus cyst of the liver. There were abundant deposits of thorium dioxide present in the liver and spleen, but no tumor formation was noted in the vicinity of these accumulations. The weight of the evidence favored the interpretation that the carcinoma was an incidental development not related to the thorium dioxide. The multicentric alveolar-cell carcinoma of the lung described by Abrahamson and co-workers [17] is also quite equivocal, since the lungs failed to show any evidence

of localization of the radioactive material by direct testing. Horta's [18] endothelial sarcoma of the liver resembled closely the lesion described by MacMahon. Horta believed that the case could be attributed to thorium dioxide despite the unusually short latent period (three years) and the unusually small dose employed (20 ml.). His studies were restricted to the liver, and no information as to lesions elsewhere was obtained. Lüdins' [19] case, reported in 1953, followed intra-arterial administration 14 years previously in which 24 ml. of thorium dioxide had been given. Focal lesions of a cystic type were encountered in the liver in which marked proliferation of reticuloendothelial cells was noted. Lüdin thought that these were hemangioendotheliomas entirely benign in their behavior. His descriptions and photographs suggest strongly that he was dealing with the same type of proliferation described by MacMahon and co-workers.

Our case parallels MacMahon's in several respects. A 14-year period of exposure was required prior to establishment of the endotheliomatous lesions. In both cases hemorrhagic cystic lesions were found scattered throughout the liver. The marked proliferation of endothelial cells together with atypical change was a prominent feature in both patients. Whether these are truly malignant neoplasms is difficult to prove in view of the absence of metastasis. The rapid course of the patient's final illness together with extensive involvement of the liver suggests that the lesions were rapidly proliferating.

The lesions encountered in the spleen have also been described by previous contributors. Here again there is extensive reticuloendothelial proliferation, but no focal lesions were noted. The eradication of lymphoid elements is in keeping with the continued exposure to irradiation from the large quantities of thorium dioxide present. There is a moderate increase in reticulum together with a generalized diffuse fibrosis. These changes are recorded in detail in the publication by Baillif,[3] who studied splenic reactions to thorium dioxide in the rat.

The local granulomatous effects of thorium dioxide are apparently a fairly frequent finding. Neoplastic transformations, however, are quite unusual. The material has been given to large numbers of patients, and in most of these no apparent noxious results have been noted. However, a long latent period is apparently necessary and follow-ups of 10 years would not be sufficient to exclude such an effect. Aub and co-workers,[20] in an excellent discussion of the late effects of internally deposited radioactive material, indicate that the average latency period in the case of radium was 23 years. There is also the additional fact, as pointed out by Orr and others [21] and by Prezyna and others,[22] that the actual radioactivity of thorium dioxide, although decreasing at first, begins to rise after 5 years, since some of the break-down products are quite active, with a peak being reached after 15 years. If these patients survive sufficiently long, other cases will undoubtedly be encountered. Diagnosis fortunately can often be suspected even if previous thorium dioxide administration is not known owing to the unusual opacity of the liver and spleen on roentgen examination.

SUMMARY

Thorium dioxide (Thorotrast) by virtue of its radioactivity has the potentiality of producing neoplastic change in man. Although such cases are rare, most patients receiving the material have not provided a sufficient time of exposure, since the latent period is longer than 10 years. A case is presented in which hemangioendotheliomatous lesions were encountered in the liver of a patient given thorium dioxide 14 years previously. Thorium dioxide-induced neoplasms in the literature include two similar cases.

REFERENCES

1. Birkner, R.: Late Injury Due to Thorotrast, Strahlentherapie **78**:587-608, 1948.

2. Thorotrast, report of the Council on Pharmacy and Chemistry, J. A. M. A. **99**:2183-2185, 1932.

3. Baillif, R. N.: Splenic Reactions to Colloidal Thorium Dioxide in the Albino Rat, Am. J. Anat. **92**:55-115, 1953.

4. Schlund, H.: Report to the Heyden Chemical Corp., New York, June 13, 1932.

5. Blühbaum T.; Frik, K., and Kalkbrenner, H.: New Colloidal Material for Roentgen Diagnosis, Fortschr. Geb. Röntgenstrahlen **37**:18-29, 1928.

6. Roussy, G.; Oberling, C., and Guérin, M.: Carcinogenic Action of Thorium Dioxide in the White Rat, Bull. Acad. méd., Paris **112**:809-816, 1934.

7. Yater, W. M., and Coe, F. O.: Ten Years' Experience with Thorotrast Hepatosplenography, Ann. Int. Med. **18**: 350-366, 1943.

8. Jacobson, L. E., and Rosenbaum, D.: Postmortem Findings and Radio-Activity Determinations 5 Years After Injection of Thorotrast, Radiology **31**:601-607, 1938.

9. Cassel, C.; Ruffin, J. M.; Reeves, R. J., and Stoddard, L. D.: Late Effects of Thorium Dioxide in Man, A. M. A. Arch. Int. Med. **88**:42-50, 1951.

10. Moeschlin, S.; Marti, H. R., and Germann, W.: Fatal Panmyelopathy Caused by Thorotrast, Schweiz. med. Wchnschr. **83**:1061-1064, 1953.

11. MacMahon, H. E.; Murphy, A. S., and Bates, M. I.: Endothelial-Cell Sarcoma of Liver Following Thorotrast Injections, Am. J. Path. **23**: 585-612, 1947.

12. Zeitlhofer, J., and Speiser, P.: Hemangioendotheliomatosis in Rabbits After Experimental Administration of Thorotrast, Ztschr. Krebsforsch. **60**:161-168, 1954.

13. Rudolphi, H.: Late Development of Carcinoma of Lower Lid Following Injections, Beitr. path. Anat. **111**:158-164, 1950.

14. Zollinger, H. U.: Spindle Cell Sarcoma of the Kidney 16 Years After Thorotrast Pyelography, Schweiz. med. Wchnschr. **79**:1266-1268, 1949.

15. Vögtlin, J., and Minder, W.: Harmful Effects of Thorotrast Following Bronchography, Retrograde Pyelography, Salpingography and Arteriography, Radiol. clin. **21**:96-115, 1952.

16. Heitmann, W.: Carcinoma of Bile Duct and Liver After Thorotrast Injection, Chirurg. **25**:223-225, 1954.

17. Abrahamson, L.; O'Connor, M. H., and Abrahamson, M. L.: Bilateral Alveolar Lung Carcinoma Associated with Injection of Thorotrast, Irish J. M. Sc., pp. 229-235 (May) 1950.

18. Horta, J. da S.: Liver Sarcoma in a Woman, Developed 3 Years and 2 Months After Thorotrast Injection, Chirurg. **24**:218-223, 1953.

19. Lüdin, M., Jr.: Hemangioendothelioma of the Liver and Spleen Caused by Thorotrast Administration, Schweiz. Ztschr. allg. Path. **16**:987-994, 1953.

20. Aub, J. C.; Evans, R. D.; Hempelmann, L. H., and Martland, H. S.: Late Effects of Internally Deposited Radioactive Materials in Man, Medicine **31**:221-329, 1952.

21. Orr, C. R.; Popoff, G. D.; Rosedale, R. S., and Stephenson, B. R.: Study of the Effect of Thorium Dioxide Sol Injected in Rabbits, Radiology **30**:370-381, 1938.

22. Prezyna, A. P.; Ayres, W. W., and Mulry, W. C.: Late Effects of Thorotrast in Tissues, Radiology **60**:573-578, 1953.

The Pathology of the Conduction System in Acquired Heart Disease

I. Severe Atrioventricular Block

MAURICE LEV, M.D.
and
PAUL N. UNGER, M.D., Miami Beach, Fla.

Since the description of the conduction system in the human heart, numerous isolated reports of studies of parts, or rarely of all, of the system in diseased states have been published. These have been reviewed in the excellent monographs of Mönckeberg [1] and Mahaim [2] and in the papers of Gross and Fried [3] and Rosenthal.[4] Recently one of us (M. L.)* has devised routine methods for the study of this system. These have been supplemented by a more thorough approach to the examination of the entire heart. The purpose of these methods was to make possible the routine study of the pathology of this system in general, and specifically of the changes in this system in various electrocardiographic disorders. The present work is the first in a series of reports of such studies.

Submitted for publication July 13, 1955.

Read before the Section on Pathology and Physiology at the 104th Annual Meeting of the American Medical Association, Atlantic City, N. J., June 8, 1955.

This investigation was supported by a grant (H-1187) from the National Heart Institute, of the National Institutes of Health, U. S. Public Health Service.

From the Research Laboratories and the Departments of Pathology and Medicine, Mount Sinai Hospital of Greater Miami, Miami Beach, and the Departments of Pathology and Medicine, University of Miami School of Medicine, Coral Gables, Fla.

* References 5 through 8.

It is not the province of this paper to discuss the controversial phases of the physiology of conduction in the human heart. This has been dealt with previously.[9] Older and more recent physiologic work would seem to support the following theses: 1. Conduction in the human heart is myogenic. 2. The impulse originates in the SA node and is transmitted to the atrial musculature which carries it to the AV node. From thence it passes successively through the AV bundle, bundle branches, to and through the ventricular myocardium. Hence, a study of the pathology of the conduction system must entail a study of (1) the SA node, (2) the approaches to the SA node, (3) the atria, (4) the approaches to the AV node, (5) the AV node, (6) the AV bundle, (7) the bundle branches throughout their extent, and (8) the ventricular myocardium. Such a clinicopathologic evaluation was undertaken by isolated section techniques by Rosenthal.[4] Mahaim's comprehensive serial section study of a large number of cases concerned itself mostly with the AV node, bundle, and bundle branches.

PATHOLOGIC METHODS

The hearts of four patients demonstrating electrocardiographic evidence of complete or severe incomplete heart block at some time in their course were studied as follows: The SA and AV nodes, the AV bundle, and bundle branches were cut and sectioned in a manner previously described.† In three hearts serial sections were done and all sections were retained, while in the fourth heart serial sections were cut and sections were retained in a manner previously described.‡ A block was then taken from the superior wall of the right atrium to include the beginning of the right circumflex

† References 5 and 7.

‡ References 5 and 7.

coronary artery and the ramus ostii cavae superiorae. This was sectioned in a serial manner and every 80th section was retained. A block was also made from the inferior wall of the right and left atria and ventricles at their point of junction (crux) to include the termination of the right (or left) circumflex, the beginning of the posterior descending coronary artery, and the beginning of the ramus septi fibrosi. This was also sectioned in a serial manner and every 80th section was retained. The anterior and posterior walls of both atria and ventricles as well as the remainder of the atrial and ventricular septa were then cut grossly, and alternating blocks were taken for section to contain a careful sampling of these tissues as well as the various valves. Single sections were cut from these blocks. This semiquantitative method of estimating pathologic change will be described more fully elsewhere. The technical details of the four cases studied are presented in Table 1.

TABLE 1.—*Technical Details of Histologic Study*

Case	Method of Sectioning Conduction System	Thickness of Section	No. of Sections: Through Conduction System	No. of Sections: Lost in Conduction System	No. of Sections: Through Remainder of Heart	Stains Used
1	Serial through SA node, AV node and bundle, and bundle branches, up to muscle of Lancisi; thereafter, q. 80	8 μ	5,916	10	133	SA node: hematoxylin-eosin and Weigert-Van Gieson. AV node, bundle, and branches to Lancisi: Weigert-Van Gieson every 5th section. Remainder hematoxylin-eosin
2	SA node, q. 20; AV node and penetrating bundle, q. 20; branching bundle and right bundle branch and left bundle branch, q. 40	6 μ	351	..	198	Alternating hematoxylin-eosin and Weigert-Van Gieson
3	Serial through SA node, AV node and bundle, and bundle branches, up to muscle of Lancisi; thereafter, q. 80	8 μ	6,185	11	192	SA node: alternating hematoxylin-eosin and Weigert-Van Gieson. Remainder: hematoxylin-eosin
4	Serial through SA node, AV node and bundle, and bundle branches up to muscle of Lancisi; thereafter, q. 80	8 μ	5,612	4	165	SA node: alternating hematoxylin-eosin and Weigert-Van Gieson. Remainder: hematoxylin-eosin

REPORT OF CASES

CASE 1.—*Clinical Summary.*—This 27-year-old white woman was first admitted to the Mount Sinai Hospital on March 12, 1948, with a history of upper respiratory infection three months prior to admission, followed by what was thought to be parotitis. Intermittently since then she had experienced dizziness, headache, severe shooting pains in the arms and legs, which responded partially to codeine. There was no history of unconsciousness, incoordination, visual defects, or other central nervous system symptoms. The past history was irrelevant. The family history revealed that the father had died of a coronary occlusion at age 61, and a brother, at 35. Physical examination showed that the patient was not acutely ill. All examinations, including neurological, were normal. The heart showed regular rhythm, rate 76 to 84. Laboratory examination revealed a red blood cell count of 4,150,000 per cubic millimeter, with 13.5 gm. of hemoglobin per 100 cc., a white cell count of 7150 with normal differential, a normal urine and spinal fluid, and a fasting blood sugar of 85 mg. per 100 cc. X-ray examination of the skull was normal. No electrocardiogram was taken at this time. The course in the hospital was entirely uneventful, and the patient was discharged two days after entry with her condition unchanged. On March 23 she was seen by a neurological consultant, who could find nothing abnormal in the nervous system despite residual radicular pains. For several months thereafter she complained of vertigo, severe migratory muscle pains, and generalized weakness. These disappeared one month before she was seen in another medical center. At this time they found nothing of note.

The patient was readmitted to the hospital on Nov. 5, 1952, four and one-half years after her first admission. She had had a history of fainting several times a year in the previous four years, and losing her equilibrium. Physical examination at this time showed that the patient was in no distress. There was no dyspnea, orthopnea, or cyanosis. The heart rate was 48 with a regular rhythm. The blood pressure was 126/56 with a questionable Grade 1 apical systolic murmur and questionably accentuated first sound. Laboratory examination showed a red blood cell count of 3,900,000 per cubic millimeter with 11.49 gm. of hemoglobin, and a

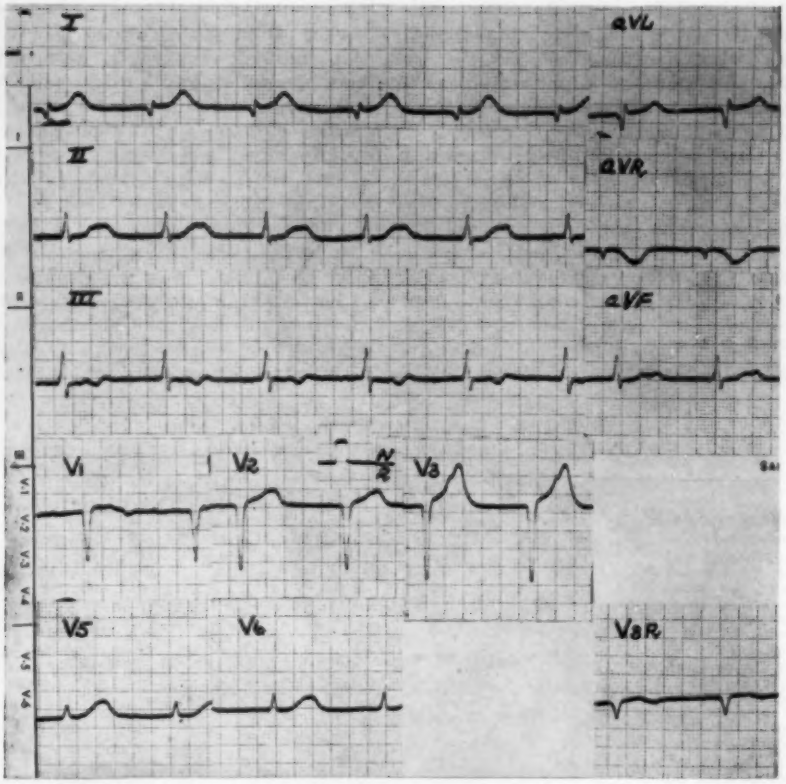


Fig. 1 (Case 1).—11/5/52. Bundle of His or bundle branch rhythm. Complete AV block. P waves low in voltage, narrow and diphasic in L_2. QRS 0.10 second. Anteroseptal damage. Prolonged Q-T interval (0.58 second).

white blood cell count of 12,500 with 69% neutrophiles, 6% band forms, 2% eosinophiles, 21% lymphocytes, and 2% monocytes. The sedimentation rate was 11, corrected, with a hematocrit reading of 42. The VDRL test was negative. The electrocardiographic examination will be discussed below. The patient was in the hospital for four days, during which time the pulse rates ranged from 44 to 80.

The patient was seen in consultation at another medical center on March 1, 1953, with an interim history, from her physician, of alternating 2:1 and complete heart block occurring in the course of a single day. Her symptoms included vertigo, faintness, and on several occasions loss of consciousness, anorexia, and anxiety. She had ineffectually received all of the sympatheticomimetic drugs and a course of cortisone. At this time the diagnosis of scleroderma was suggested.

The patient was readmitted on April 4, 1953, for digitalization, in an effort to produce constant and complete AV block, since it was felt that she had most of her difficulty when her block varied. On physical examination the patient was not acutely ill. There were no findings other than those referable to the cardiovascular system. The heart showed a rate of 62 with an irregular rhythm. There was a Grade 1-2 nontransmitted apical systolic murmur. The blood pressure was 128/78. The patient was digitalized with digoxin, receiving 3 mg. by mouth in 24 hours, and was maintained on 0.5 mg. daily during the remainder of her stay, with no success in establishing complete heart block or alleviating her symptoms. The pulse rates varied from 42 to 72, with rates below 60 most of the time. She was discharged on a maintenance dose of 0.5 mg. of digoxin eight days after admission. Six days later the patient died suddenly.

Electrocardiographic Data.—Nov. 4, 1952: Second-degree heart block with 3:1 response and P-R interval of conducted impulses of 0.42 second. The P waves were of low voltage throughout. The QRS duration was 0.10 second. A QS deflection was noted in V_1; *q*R in V_2, V_5, and V_6. Septal Q waves were absent from V_5, V_6, and aV_L. The T waves were very broad and notched, with terminal

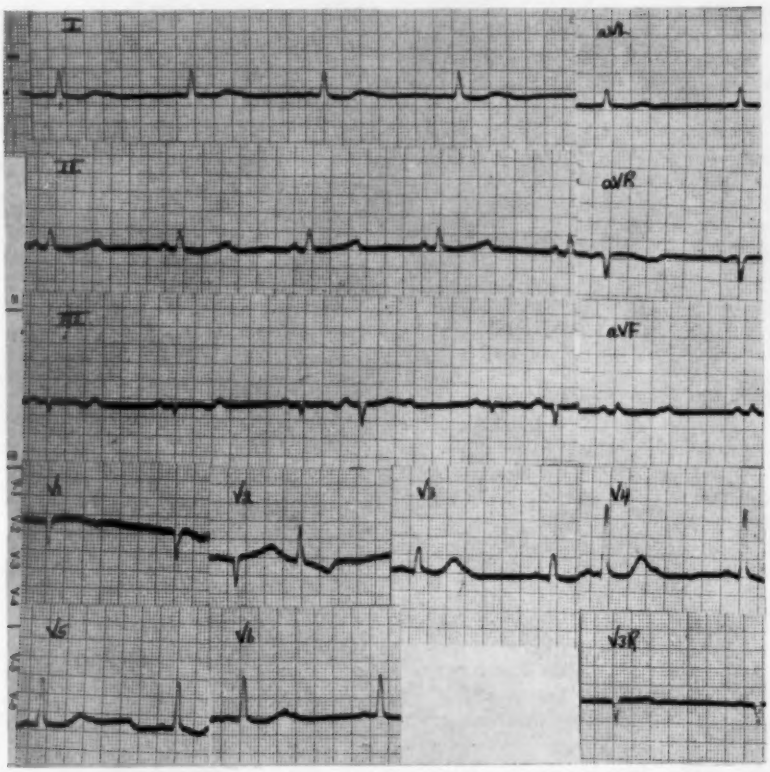


Fig. 2 (Case 1).—4/27/53. Sinus rhythm. First- and second-degree AV block, mainly latter. Occasional nodal and ventricular extrasystoles. Incomplete left bundle branch block. Anteroseptal damage. Prolonged Q-T interval (0.60 second).

negativity in V_2 and V_3 and minus-plus in aV_L. The Q-T duration was 0.66 second which, with an R-R interval of 1.66 seconds, is abnormal for this rate.

Nov. 5, 1952 (Fig. 1): Complete AV block. P-P interval of 0.46 second, rate of 130. P waves were low in voltage, very narrow, and diphasic in lead II. QRS duration was 0.10 second. QRS complex was of supraventricular configuration and could be arising in middle portion of the node, in which instance P waves were obscured, or it may have originated in other portions of the AV node with retrograde block. It may also possibly have originated in the bundle of His or bundle branches. Abnormal Q wave in lead I, aV_L, and QS deflections in V_{2R} and V_1 through V_3 with absent septal Q in V_5 and V_6 indicated anteroseptal damage and/or left bundle branch block. Q-T interval was 0.58 second which, with R-R interval of 1.14 seconds, is abnormal for this rate.

Nov. 6, 1952: Second-degree 2:1 AV block. P waves as in previous tracing. Incomplete left bundle branch block and/or damage to septum. Absent Q in lead I, aV_L, V_5-V_6. R was slurred. qR in V_{2R} and V_1. Q-T was 0.60 second which, with R-R interval of 1.24 seconds, is abnormal for this rate.

Nov. 8, 1952: Continuous lead II tracing showed second-degree heart block with 2:1 response and P-R of conducted impulses of 0.20 second. Q-T duration was 0.56 second, with an R-R of 1.36 seconds. The P waves were of low voltage notched and variable in form and height, with complexes appearing to arise in the SA node. Other P waves were of minus-plus type, with low voltage and a different P-R interval from those mentioned previously, and originating at a somewhat more rapid rate. These resembled complexes arising in auricular muscle with wandering of the pacemaker between the SA node and within the SA node to the auricles. The QRS duration was 0.10 second, and the complexes resembled those seen on the previous tracing.

Jan. 12, 1953: Variations from normal P-R interval to 2:1 heart block. The P waves were of low voltage. The R was slurred with a duration of QRS of 0.10 second. V_2 and V_3 had changed from a qR on tracing of Nov. 4, 1952, to a QS. Septal Q

waves were still absent in V_5, V_6, and aV_L. U waves were noted. The Q-T duration, when heart block was absent, was 0.44 second, with R-R of 0.80 second, which is abnormal for this rate. Where second-degree 2:1 AV block was present, the Q-T was 0.52 second for R-R of 1.30 seconds, which is abnormal for this rate. Another long lead II, taken on March 25, 1953, showed second-degree heart block with 3:1, 2:1, and occasionally 1:1 response. The P-R of all conducted impulses was 0.16 second. The P waves were notched. QRS was 0.10 second with slurring of R.

April 24, 1953: First- and second-degree heart block. P waves of sinus origin. Again showed incomplete left bundle branch block and/or septal damage.

April 27, 1953 (Fig. 2): First- and second-degree AV block, mainly the latter. Occasional nodal and ventricular extrasystoles. P waves were of sinus origin and, except for borderline voltage, were of normal configuration. Incomplete left bundle branch block and/or septal damage. QRS duration 0.10 second and slurred R. Q-T duration was 0.62 second which, with R-R interval of 1.40 seconds, is abnormal for this rate.

Electrocardiographic Interpretation.—In summary, this series of electrocardiographs showed evidence of impaired AV conduction, auricular damage, incomplete left bundle branch block, and damage to the left ventricle and interventricular septum.

Clinical Diagnosis.—Heart block with ventricular arrest, cause undetermined.

POSTMORTEM FINDINGS

Aside from the findings in the heart, the pathologic diagnosis was: (1) chronic, passive hyperemia of the lungs with fibrosis and (2) acute degeneration of septa of lung.

HEART

Gross Examination.—The heart weighed 290 gm. The endocardium of the right atrium in the region of the AV node presented a few dilated vessels, and it was slightly puckered. The left ventricle was slightly increased in thickness. The coronary artery showed a considerable number of sclerotic plaques for this age, but there was no narrowing. The aorta and the mitral valve likewise presented occasional plaques. There were no further changes.

Microscopic Examination.—The endocardium, pericardium, and the valves presented no remarkable changes.

Myocardium: Left ventricle. There was a diffuse fine proliferation of young connective tissue, with varying amounts of collagen, with an accentuation of the process beneath the endocardium (Fig. 3). This tissue was especially prominent around capillaries and venules. Within this connective tissue there were zones of dissolution of collagen with a scattering of macrophages and lymphoid cells (Fig. 4). In the subendocardial region the adventitial connective tissue was concentrically increased, and there was some compression of vessels. In addition, some arterioles showed local subintimal thickenings. Others presented acute degenerative changes with foci of degeneration in the adventitial perivascular collagen. The myocardial fibers were focally enlarged, and many were deeply stained eosinophilically. This was especially noticeable subendocardially.

Right ventricle. The fibrotic process was somewhat less marked, but the acute vascular and fiber degeneration were the same.

Ventricular septum. Here all the above myocardial changes were maximal.

Left and right atria. The fibrotic process was present, but less marked than in the ventricles.

Atrial septum. This was involved to the same degree as the atria.

Conduction System: SA node. The arterioles in the node showed thickening of the adventitia, and some were narrowed. The node itself, however, showed no significant changes.

Approaches to the SA node. There was a slight proliferation of young connective tissue with focal infiltration of lymphoid cells. Some of the muscle fibers were enlarged, and some showed acute degenerative changes. Many arterioles showed concentric thickening of the adventitia.

Approaches to the AV node. There was marked fatty infiltration of the approaches to the node with a proliferation of young connective tissue and a scattering of lymphoid and plasma cells (Figs. 5 and 6). Some of the muscle fibers showed acute degenera-

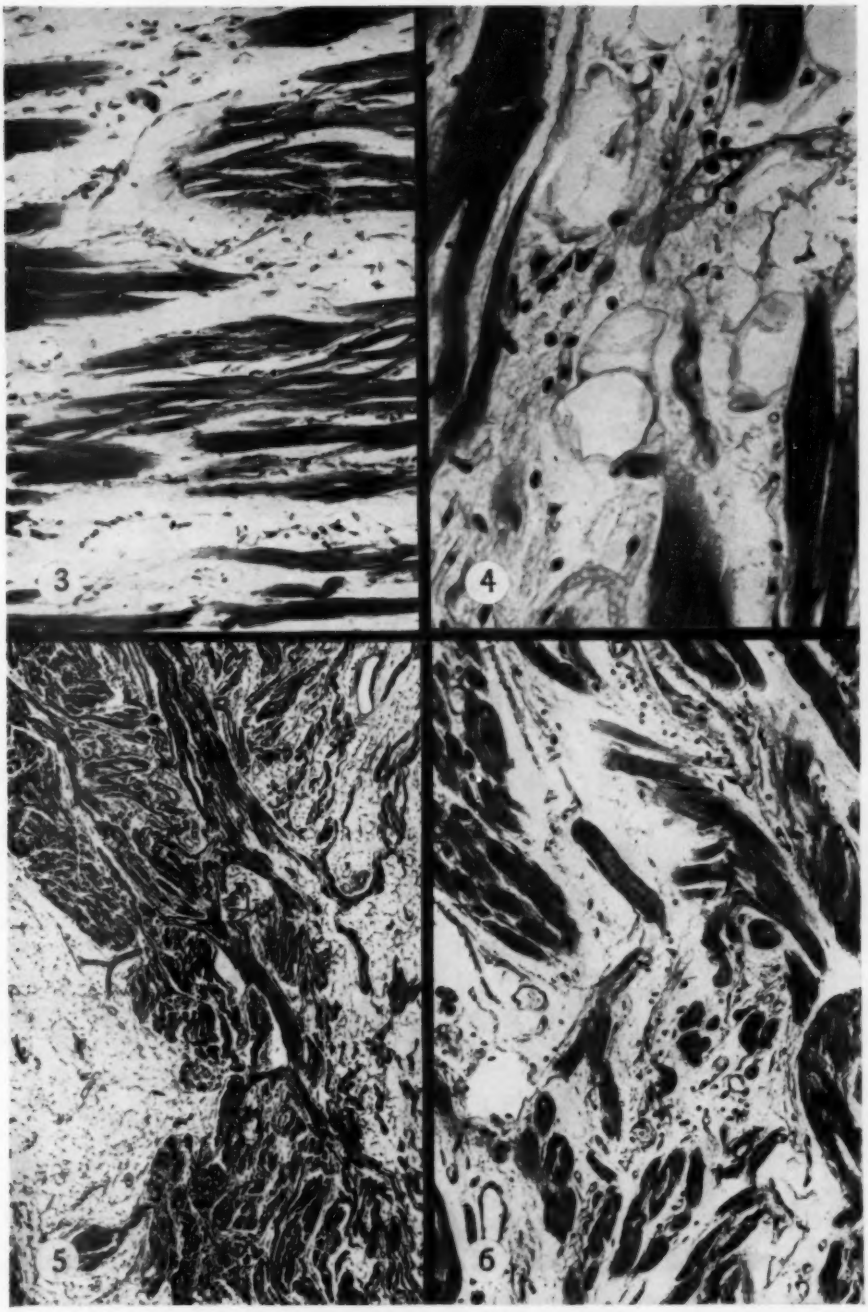


Fig. 3 (Case 1).—Section of myocardium showing a diffuse increase in connective tissue, with degenerative changes in collagen and muscle fibers. Hematoxylin-eosin stain; × 150.

Fig. 4 (Case 1).—Section of myocardium showing fibrosis, fatty infiltration, and an infiltration of lymphoid cells. Hematoxylin-eosin stain; × 300.

Fig. 5 (Case 1).—Section of the approaches to the AV node showing fatty infiltration. Hematoxylin-eosin stain; × 30.

Fig. 6 (Case 1).—Section of the approaches to the AV node showing fibrosis with an infiltration of lymphoid cells. Hematoxylin-eosin stain; × 150.

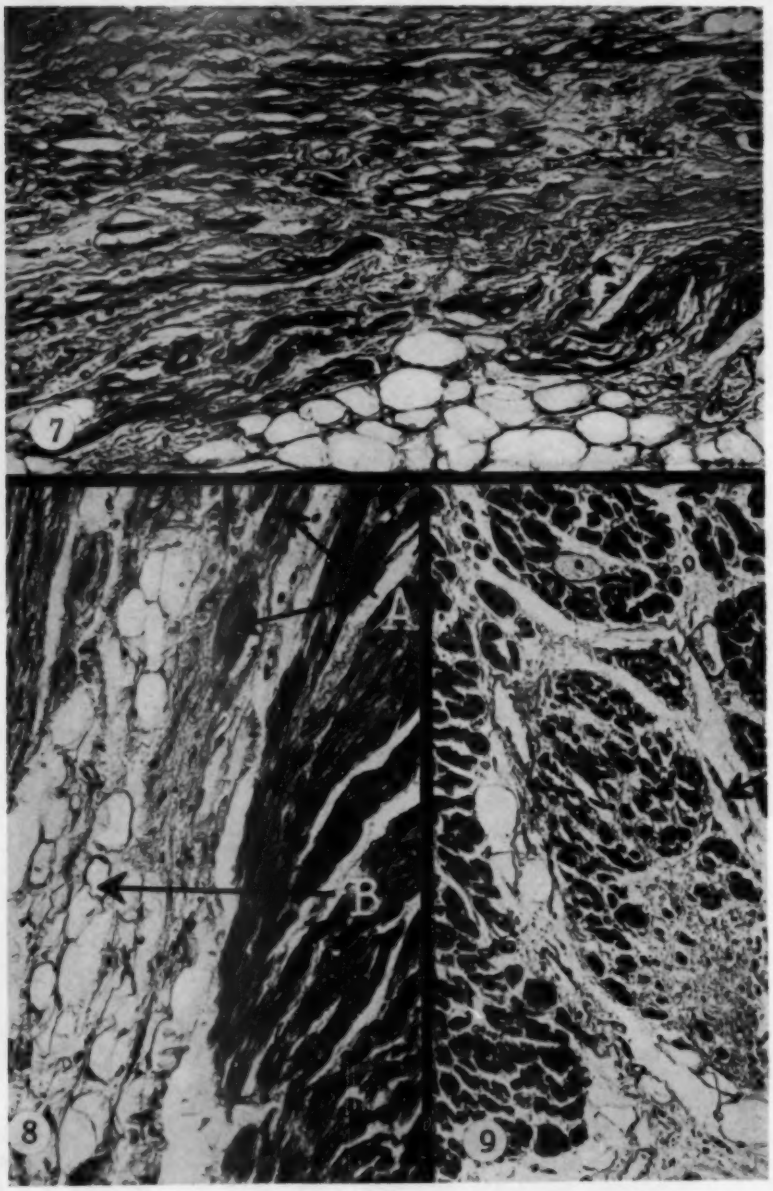


Fig. 7 (Case 1).—Section of the AV node showing marked fibrosis. Hematoxylin-eosin stain; × 150.

Fig. 8 (Case 1).—Section of the upper part of the left bundle branch showing fatty infiltration, fibrosis, and replacement of left bundle branch. Hematoxylin-eosin stain; × 150.

A, remaining fibers of the left bundle branch; *B*, area of replaced fibers.

Fig. 9 (Case 1).—Section of right bundle branch about two-thirds of its distance downward, showing partial replacement of collagen and elastic fibers. Hematoxylin-eosin stain; × 150.

Arrow points to the right bundle branch.

tive changes. It was evident that although muscular continuity with the atrium was maintained, the muscular connections were diminished and compromised.

AV node. The initial segment of the node showed a marked proliferation of fibrous connective tissue with partial replacement of nodal tissue (Fig. 7). There was a slight infiltration of lymphoid cells, with some irregularity in staining of nodal fibers. Some arterioles in the node showed acute degenerative change.

AV bundle. Both the penetrating and branching portions showed slight irregularly increased eosinophilic staining of fibers.

Left bundle branch. This showed in the upper portions areas of fatty infiltration and replacement of fibers by connective tissue (Fig. 8). The collagen showed focal dissolution with occasional infiltration of mononuclear cells. There were moderate acute degenerative changes in some of the muscle fibers.

Right bundle branch. In the first part of its course there was fatty infiltration in excess for this age. In areas of infiltration there were acute degenerative changes in some muscle fibers. Distal to the muscle of Lancisi, up to the moderator band, there was a focal increase in connective tissue in the right branch (Fig. 9). At no point, however, was the complete continuity of the branch destroyed.

Blood supply to the SA and AV nodes. Both the ramus septi fibrosi and the ramus ostii cavae superiorae showed no change.

PATHOLOGIC DIAGNOSIS—CASE 1

1. Marked fibrosis, with slight chronic inflammation of the myocardium with recent vascular and collagen degeneration. (Chronic myocarditis? Scleroderma-like heart disease?)
   (*a*) Fibrosis, with chronic inflammation and fatty infiltration of the approaches to the AV node
   (*b*) Marked fibrosis of the beginning of the AV node
   (*c*) Focal fibrosis and fatty infiltration of the right and left bundle branches
   (*d*) Mild acute degenerative changes of the AV node and bundle and right bundle branch
   (*e*) Moderate acute degenerative changes of the left bundle branch

CLINICOPATHOLOGICAL SUMMARY (TABLE 2)

Case 1 was a case of scleroderma-like heart disease or chronic myocarditis with marked fibrosis in a young woman, with changes in the conduction system which had produced, clinically, heart block varying from complete to incomplete, with left bundle branch block, present for five to six months. These pathologic changes were: (1) fibrosis with chronic inflammation and fatty infiltration of the approaches to the AV node, and (2) fibrosis of the beginning of the AV node and focal fibrosis and fatty infiltration of the left and right bundle branches. The patient proceeded to develop progressive fibrotic changes in the left ventricle and the septum and died either in a Stokes-Adams episode or in left ventricular failure.

TABLE 2.—*Case 1—Clinicopathologic Correlation*

	Electrocardiographic Interpretation		Pathologic Changes in Conduction System
1	Complete varying with partial AV block	1	Fibrosis, chronic inflammation, fatty infiltration to approaches to AV node, and fibrosis of beginning of node
2	Incomplete left bundle branch block	2	Fibrosis, fatty infiltration of left bundle branch
3	.....	3	Fibrosis, fatty infiltration of right bundle branch

CASE 2.—*Clinical Summary.*—This 30-year-old white woman was admitted to another hospital on July 23, 1954, with complaints of headache, nausea, retching, weakness, generalized malaise, and repeated "black-out" spells of one day's duration. The previous and family histories were noncontributory. There was no previous history of cardiac or renal disease.

Physical examination revealed a well-nourished young woman in acute distress. Temperature 103.8 F, and respiration rate 20. The head, neck, and lungs were negative. The heart was not enlarged. No murmurs were heard. The pulse and ventricular rates were regular, equal, and 90. There was generalized tenderness of all the muscles of the body. The liver was enlarged and tender. The spleen was not felt. All deep reflexes tested were normal.

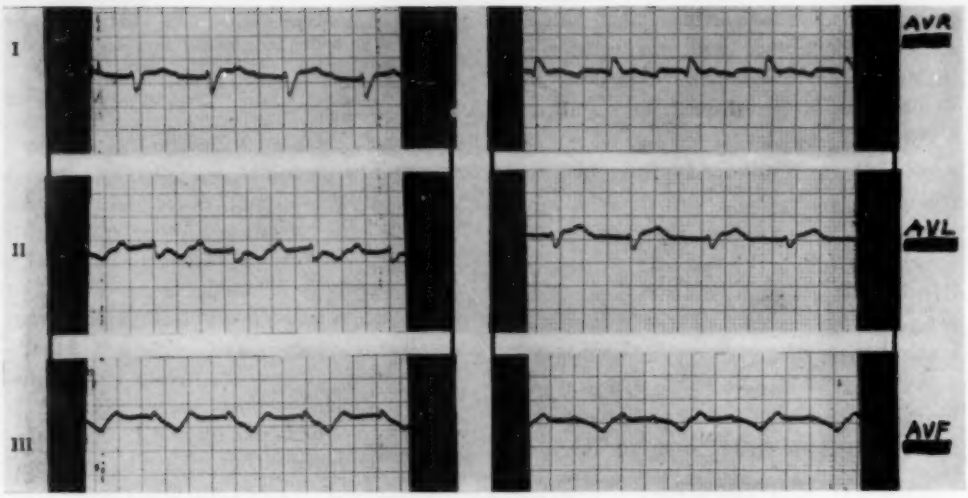


Fig. 10 (Case 2).—7/25/54. Standard limb leads and unipolar extremity leads. First-degree AV block. P-R interval 0.34 second. P waves broad and notched. QRS 0.12 second. Complete right bundle branch block. Sinus rhythm.

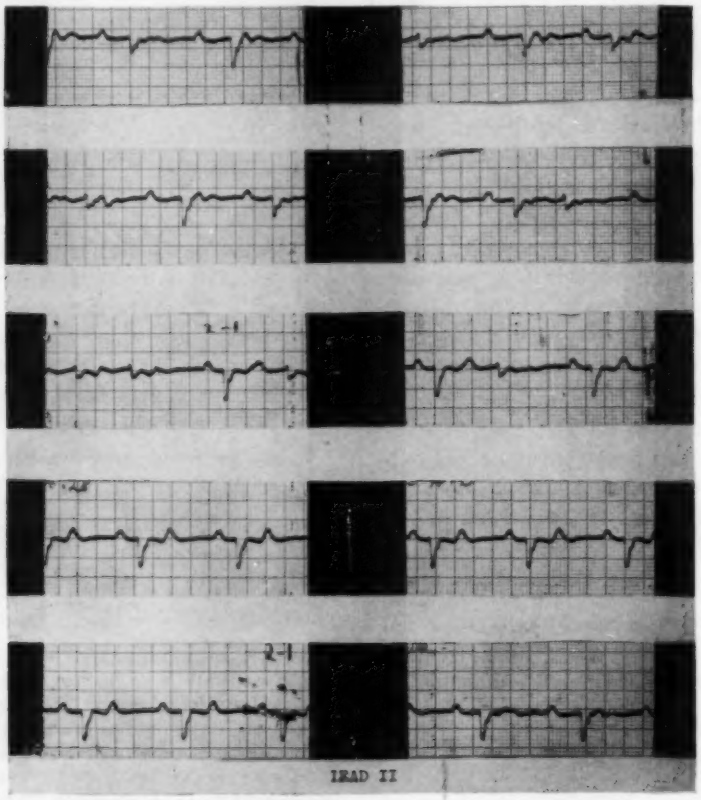


Fig. 11 (Case 2).—7/25/54. L_2 only. First- and second-degree heart block. Intraventricular conduction defect. Sinus rhythm.

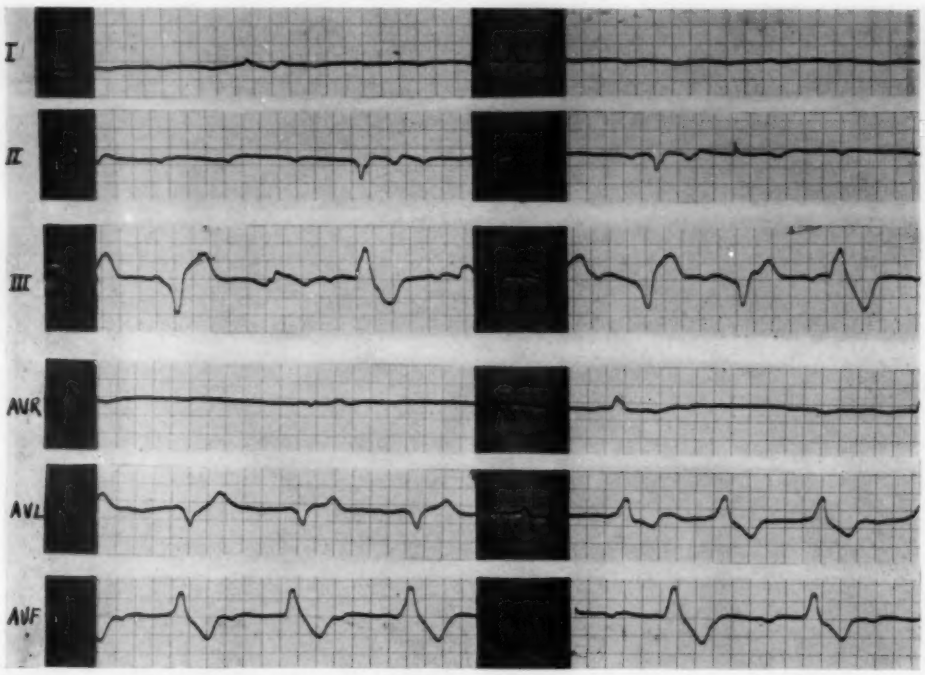


Fig. 12 (Case 2).—7/28/54. AV nodal rhythm. Complete heart block with multifocal ventricular complexes of monophasic type.

Laboratory examination revealed the spinal fluid to be normal. The red blood cell count, on admission, was 4,500,000 per cubic millimeter, with a hemoglobin of 85%. The white blood cell count was 11,500 per cubic millimeter, with 7% stab forms, 55% neutrophiles, 35% lymphocytes, and 3% monocytes. The urine showed a trace of albumin, 2 to 4 WBC and 20 to 40 RBC per high-power field. Electrocardiographic examination will be discussed below.

The patient developed marked dyspnea and convulsive seizures, with ventricular rates of 16 noted prior to the seizures. At one point these seizures occurred every 15 minutes. The temperature ranged from 99 F to 103.8 F in the first 24 hours, from 99.6 F to 101.0 F the second 24 hours, and rose to 103.6 F at time of death. The patient went into shock with repeated and almost continuous emesis of coffee-ground, dark brown material. She then went into coma with repeated convulsions and died.

Electrocardiographic Data.—July 25, 1954, Day of Admission (Fig. 10): First-degree heart block. P-R interval 0.34 second, QRS 0.12 second. Right bundle branch block, complete, and complicated by septal damage (shown in precordial leads). P-P interval 0.68 second equaled rate of 88, regular sinus rhythm. P waves were broad and notched.

Lead II only, later same day (Fig. 11). First- and second-degree heart block with variation in magnitude of similar QRS complexes, depending upon P-R interval of conducted impulses. It was noted that where P-R interval of conducted impulses was 0.40 second, the QRS complex was of greater voltage than those which followed P-R interval of 0.24 second.

July 26: Complete heart block with P waves resembling those seen on tracings of previous day and arising in the SA node. P-P interval 0.44 second equaled rate of 136, with periods of ventricular asystole and ventricular multifocal contractions, single and multiple. The precordial leads showed periods of auricular asystole in addition to the preceding.

July 28 (Fig. 12): Showed change in P wave voltage and configuration. Analysis of leads II and III showed negative P waves (probably AV nodal in origin) with P-P interval of 0.76 equaled rate of 79. Complete heart block was present, with multifocal ventricular complexes of monophasic type.

July 28, Lead III only: Showed complete heart block with multifocal ventricular impulses and idioventricular rhythm. P waves occur inconstantly, some upright and some negative, indicating multi-

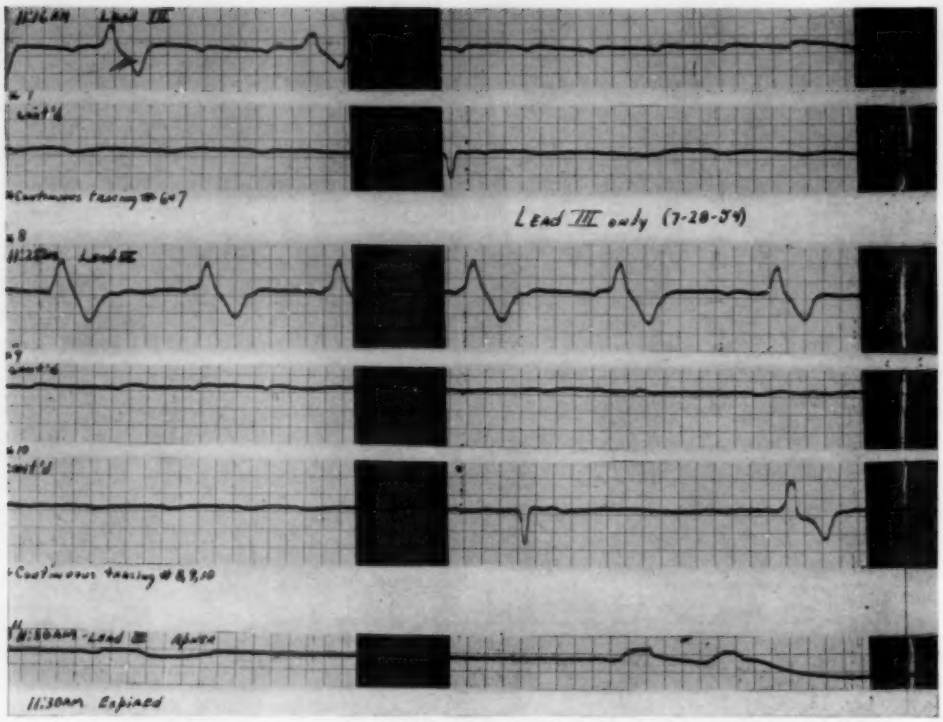


Fig. 13 (Case 2).—7/28/54. L_3 only. Complete heart block. Irregular inconstant, monophasic ventricular complexes with ultimate ventricular arrest preceded by auricular arrest.

focal origin either in SA node, auricle, or AV node. At times no evidence of auricular activity was seen.

July 28, Lead III only (Fig. 13): Portions from a continuous tracing taken over course of 25 minutes until ventricular arrest occurred. In line 1 regular supraventricular activity with P-P interval of 0.74 second, rate of 81, has reappeared. This disappeared in line 2 and reappeared irregularly in the following lines. Complete heart block was present with monophasic and inconstant irregular ventricular activation, with no ventricular activity in the last line.

Electrocardiographic Interpretation.—In summary, this electrocardiographic series showed initially the development of first-degree heart block, evidence of auricular damage, right bundle branch block, ventricular septal damage, and damage to other portions of the left ventricular muscle. This proceeded to second-degree heart block and to third-degree or complete AV block. When the latter occurred, the idioventricular rhythm did not permit the further recognition of bundle system block. On the final day of the patient's life the auricles were activated from varying foci—at times the AV node, and at other times the SA node, with marked variability in the auricular complexes, leading terminally to absent auricular activity before ventricular activity ceased. There was thus damage to the auricles, the AV bundle and/or its approaches, the right bundle branch, the ventricular septum, and the left ventricle. When complete heart block developed, the idioventricular rhythm prohibited the recognition of any underlying damage to the right or left bundle branches.

Clinical Diagnosis.—Acute myocarditis, cause undetermined.

POSTMORTEM FINDINGS

Apart from findings in the heart, the pathologic diagnosis was: (1) central and midzonal necrosis of the liver, (2) acute splenic hyperplasia with follicular necrosis, (3) severe cloudy swelling of the kidneys, and (4) recent thrombus in the adrenal vein.

HEART

Gross Examination.—The heart weighed 240 gm. The epicardial surface of the left atrium, the endocardial surface of the right

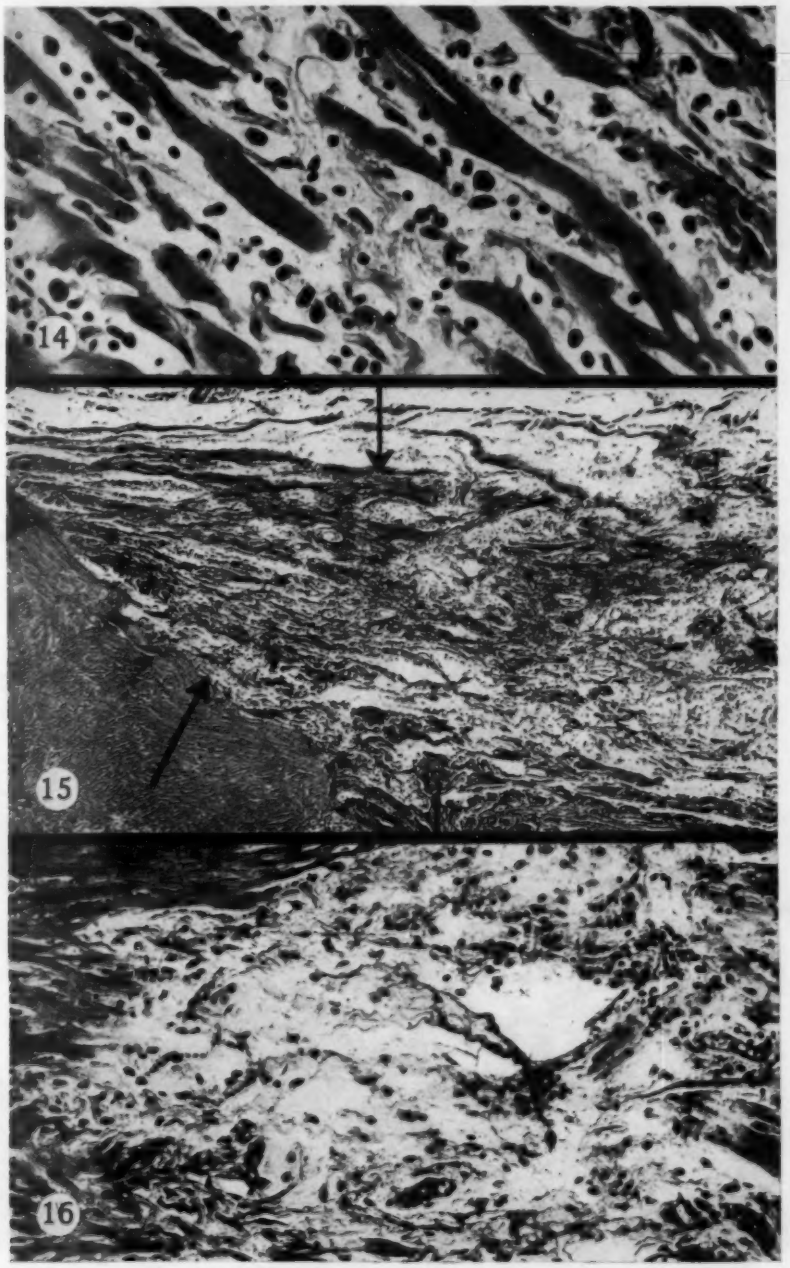


Fig. 14 (Case 2).—Section of the ventricular myocardium showing acute myocarditis with mononuclear cells. Hematoxylin-eosin stain; × 300.

Fig. 15 (Case 2).—Topographic view of the AV node. Hematoxylin-eosin stain; × 30. Arrows point to the node.

Fig. 16 (Case 2)—Section of the AV node showing an infiltration of mononuclear cells and proliferation of sheath cells. Hematoxylin-eosin stain; × 150.

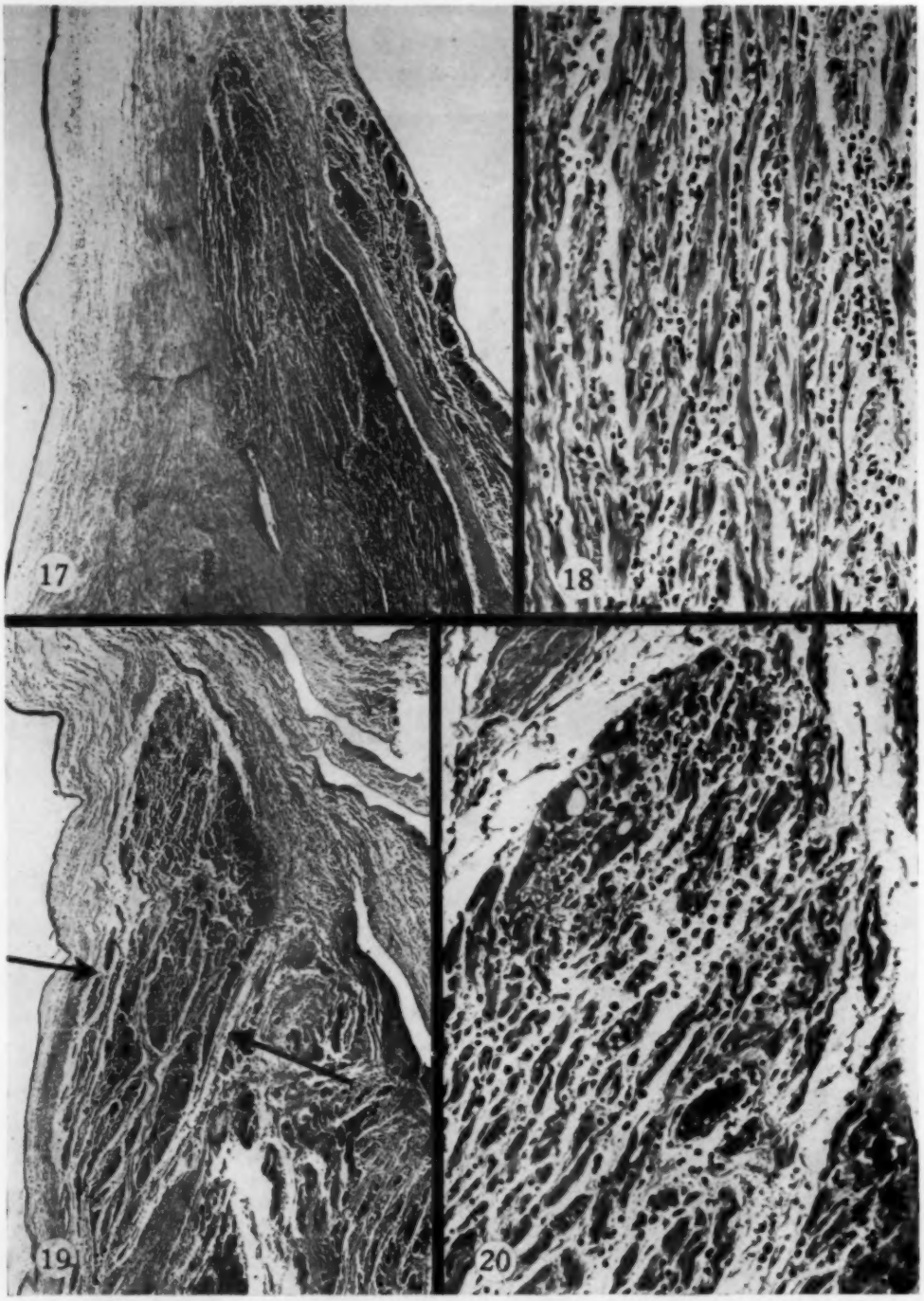


Fig. 17 (Case 2).—Topographic view of the end of the AV node and the beginning of the penetrating portion of the AV bundle. Hematoxylin-eosin stain; × 30.

Fig. 18 (Case 2).—Section of the penetrating portion of the AV bundle showing a marked infiltration of lymphoid cells. Hematoxylin-eosin stain; × 150.

Fig. 19 (Case 2).—Topographic view of the branching portion of the AV bundle. Hematoxylin-eosin stain; × 30.

Arrows point to the bundle.

Fig. 20 (Case 2).—Section of the branching portion of the AV bundle showing a marked infiltration of lymphoid cells, with necrosis of some fibers. Hematoxylin-eosin stain; × 150.

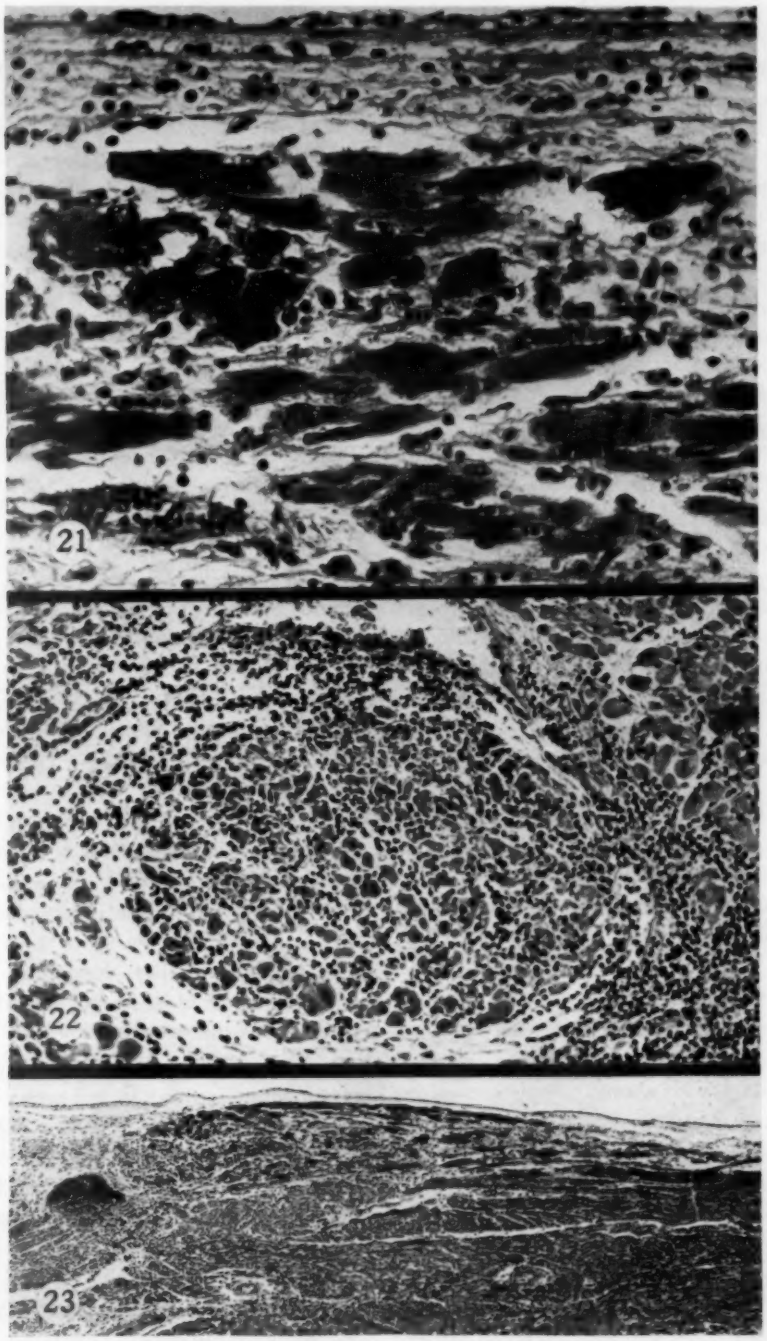


Fig. 21 (Case 2).—Section of the left bundle branch showing almost complete necrosis. Hematoxylin-eosin stain; × 300.

Fig. 22 (Case 2).—Section of the right bundle branch in its midportion showing a marked infiltration of lymphoid cells with focal necrosis. Hematoxylin-eosin stain; × 150.

Fig. 23 (Case 2).—Topographic view of end of right bundle branch showing marked inflammation with necrosis. Hematoxylin-eosin stain; × 30.

atrium and ventricle, and especially the endocardial covering of the left bundle branch presented petechial hemorrhages. At the site of the SA node an area of slight puckering and tannish discoloration was seen. The entire cardiac musculature was pale brownish-tan, parboiled in appearance, and very flabby. There were no further changes.

Microscopic Examination.—Pericardium: This was slightly to moderately infiltrated with mononuclear cells with degenerative changes in the subepicardial fat.

Endocardium: This was also focally infiltrated with mononuclear cells. The Purkinje fibers will be described below.

Valves: The collagen of the valves showed focal areas of degeneration with a slight infiltration of mononuclear cells.

Myocardium: Left ventricle. The myocardium was markedly involved in an inflammatory and necrotic process (Fig. 14). The myocardial fibers showed marked degenerative and necrotic changes. The interstitial tissue was markedly infiltrated with lymphoid cells, plasma cells, and macrophages. No neutrophiles were noted. The collagen fibers showed destructive changes. The adventitia and periadventitial connective tissue were involved in the process, but the media and intima of the arteries were relatively not involved.

Right ventricle. The process here was not so marked as in the left ventricle.

Ventricular septum. Here the process was maximal, with almost complete involvement of the septum.

Right and left atria and atrial septum. The inflammatory and necrotic process here was mild and focal in comparison with that of the ventricles.

Conduction System: SA node. Some of the muscle fibers show marked eosinophilic staining with or without pyknotic nuclei. This, however, was a relatively mild process as compared with the process in the approaches.

Approaches to the SA node. The process described in the remainder of the myocardium was seen here and was severer than that in the remainder of the atria. This involved Wenckebach's bundle, Bachmann's bundle, and the atrial appendage.

Approaches to the AV node. The process here was the same in amount as that at the approaches to the SA node and severer than that in the remainder of the atria. There was a marked proliferation of sheath cells just adjacent to the origin of the AV node.

AV node. There was a marked infiltration of mononuclear cells with a marked proliferation of sheath cells (Figs. 15 and 16). The muscle fibers showed some degenerative, but no necrotic, changes.

AV bundle. The penetrating portion showed a marked infiltration of mononuclear cells with moderate degenerative, but no necrotic, changes (Figs. 17 and 18). The branching portion of the bundle, however, showed distinct necrotic changes in addition (Figs. 19 and 20). This became progressively more marked distally up to and including the region of bifurcation. However, this was not so marked as that found in the left bundle branch.

Left bundle branch. This was completely involved in the necrotic and inflammatory process described in the myocardium (Fig. 21). Some of the Purkinje cells showed eosinophilic and others basophilic necrosis. This process extended throughout the entire ramifications up to the papillary muscles.

Right bundle branch. At the origin there was moderate infiltration and necrosis, which appeared to become less up to the region of the muscle of Lancisi. Distal to this point the changes increased (Fig. 22). The terminal portion in the region of the moderator band showed complete necrosis and a marked infiltration of cells (Fig. 23).

Blood supply to the SA and AV nodes. The ramus ostii cavae superiorae and the ramus septi fibrosi showed no changes. The small arteries and arterioles in the region of the SA and AV nodes showed the same changes as those described in the myocardium.

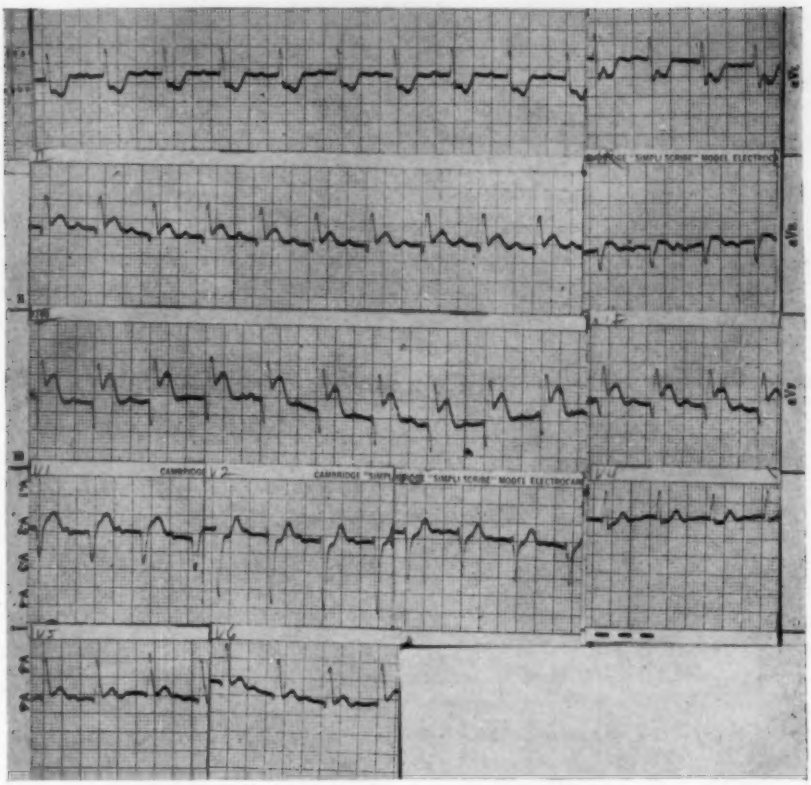


Fig. 24 (Case 3).—10/2/51. First-degree AV block. Sinus rhythm. QRS 0.12 second. Complete left bundle branch block. Acute posteroseptal myocardial infarction.

PATHOLOGIC DIAGNOSIS

1. Acute diffuse myocarditis, with involvement of the conduction system:
   (*a*) Minimal changes in the SA node
   (*b*) Moderate changes in the approaches to the SA and AV nodes
   (*c*) Moderate changes in the AV node and penetrating portion of bundle
   (*d*) Moderate to marked changes in the branching bundle and right bundle branch
   (*e*) Maximal change in the left bundle branch

CLINICOPATHOLOGICAL SUMMARY (TABLE 3)

Case 2 was a case of acute diffuse myocarditis, occurring in a young woman, with marked involvement of the left ventricle, especially of ventricular septum, less involvement of the right ventricle and atria, and marked involvement of the conduction system.

Electrocardiographically, this patient showed partial heart block with right bundle branch block progressing to complete heart block, with varying sinus and nodal rhythm. There was also evidence of atrial and ventricular damage. The changes can be correlated with inflammatory and necrotic changes in the approaches to the SA and AV nodes and the entire conduction system. The maximal necrosis and the probable cause of the heart block, however, appear to have been at the terminal portion of the bundle. The complete necrosis of the left bundle

TABLE 3.—*Case 2—Clinicopathologic Correlation*

	Electrocardiographic Interpretation		Pathologic Changes in Conduction System
1	Partial, progressing rapidly to complete heart block	1	Necrosis of terminal part of AV bundle, and left and right bundle branches
2	Sinus rhythm predominant, and occasional run of nodal rhythm	2	Inflammation of AV node and approaches to SA node
3	Right bundle branch block, early	3	Necrosis of right bundle branch

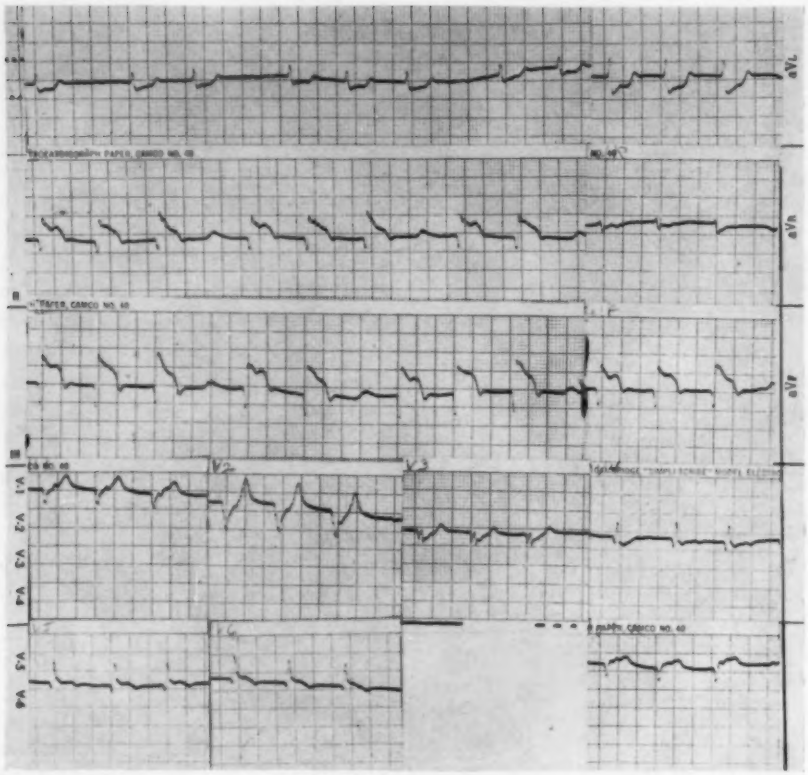


Fig. 25 (Case 3).—10/3/51. Sinus rhythm. First- and second-degree heart block. Broad notched P waves. Complete left bundle branch block. Extension of infarct to anterior wall and free walls of the right and left ventricles.

branch was clinically obscured in the presence of complete heart block and idioventricular rhythm.

Case 3.—*Clinical Summary.*—This 60-year-old white man entered the Mount Sinai Hospital with a history of precordial pain after meals and effort for several months and excruciating precordial pain radiating to both shoulders and arms of a half-hour duration. There was no previous history of diabetes, hypertension, or renal disease. Physical examination revealed the patient to be in acute distress, with ashen cyanosis and marked dyspnea. The blood pressure was 170/?. The heart was not enlarged. The heart tones were distant, with an irregular ventricular rate of 50. The left pupil was markedly smaller than the right, and both reacted to light. Laboratory examination revealed a red blood cell count of 5,290,000 per cubic millimeter, with 16.8 gm. of hemoglobin per 100 cc. The white blood cell count was 27,300, with 80% neutrophiles, 12% lymphocytes, 7% stab forms, and 1% eosinophiles. Electrocardiographic examination will be discussed below. The patient remained in shock and oliguria for 26 hours and died, despite vasoconstrictors, intra-arterial transfusion, and other routine supportive therapy.

Electrocardiographic Data.—Oct. 2, 1951 (Fig. 24): Acute posteroseptal myocardial infarction. First-degree AV block. P-R interval 0.24 second. P-P interval 0.64 second equaled rate of 94 of sinus origin. QRS duration of 0.12 second, complete left bundle branch block.

Oct. 3, 1951 (Fig. 25): First- and second-degree heart block. P-R interval 0.36 second to 0.44 second with dropped beats. Rhythm arose in SA node. P waves became broad and notched. Complete left bundle branch block persisted. The infarct involved, in addition to the posterior wall of the left ventricle, the anterior wall and the free walls of the right ventricle and the left ventricle.

Oct. 3, 1951: First- and second-degree heart block, but auricular rate and P-R interval could not be accurately determined from this tracing. P waves were of sinus origin. Ventricular rate irregular with QRS complexes of supraventricular type. This tracing was incomplete and the leads not

long enough for satisfactory measurement and complete evaluation.

Electrocardiographic Interpretation.—Damage had occurred to the auricles, the conductive system involving either the approaches to the AV node or the AV node itself, the bundle, the left bundle branch and the ventricular myocardium, predominantly the septum and posterior wall, also the anterior wall of the left ventricle, and the free walls of the left and right ventricles at the apical region of the heart.

Clinical Diagnosis.—Arteriosclerotic heart disease with coronary thrombosis with acute myocardial infarction.

POSTMORTEM FINDINGS

Aside from the findings in the heart, the pathologic diagnosis was: (1) pulmonary edema, (2) passive hyperemia of the liver, (3) fibrosis of the spleen, (4) senile sclerosis of the kidneys, and (5) focal hemorrhagic necrosis of the large intestine.

HEART

Gross Examination.—The heart weighed 400 gm. The left ventricle was moderately hypertrophied. The epicardium over the anterior surface of the left ventricle presented numerous red dots. The myocardium of the left ventricle presented numerous red areas in the septal, anterior, and posterior walls of the left ventricle. The posterior wall of the left ventricle presented, in addition, a grayish-white scar at the base. The coronary arteries showed numerous plaques, which had narrowed the anterior descending and left circumflex branches. In addition, a fresh thrombus occluded the anterior descending, and another thrombus narrowed the right main circumflex, close to its origin. There were no further changes.

Microscopic Examination. — Epicardium and Endocardium: These showed foci of infiltration of mononuclear cells. In addition, the epicardium showed a focal proliferation of fibroblasts.

Valves: These showed the usual aging changes.

Myocardium: Left ventricle. The entire musculature of the left ventricle was involved in an acute and organizing infarct. In addition, there were areas of old infarction in the anterior and posterior walls.

Right ventricle. The myocardium here likewise showed a considerably sized acute infarct involving the anterior and posterior walls. In addition, there were small microscopic scars in both walls.

Ventricular septum. This also showed almost complete involvement in an acute infarct with areas of fibrosis.

Right and left atria and atrial septum. These also showed large areas of recent and organizing infarction with areas of fibrosis.

Conduction System: SA node. This showed no remarkable changes.

Approaches to the SA node. There was considerable fibrosis of the atrial appendage adjacent to the node, the posterior crest, Bachmann's bundle, and Wenckebach's bundle. In addition, there were small recent and organizing infarcts in these regions (Fig. 26).

Approaches to the AV node. A recent infarct was present at the medial and inferior approaches. There were also areas of fibrosis.

AV node. There were acute degenerative changes in some of the muscle fibers (Figs. 27 and 28).

AV bundle. The penetrating portion showed the same focal degenerative changes as the node. The branching portion, however, showed more widespread acute degenerative changes. Both branching and penetrating portions showed an increase in connective tissue (Fig. 29).

Left bundle branch. From the beginning down, the left bundle branch shows early necrotic change. Some Purkinje fibers were markedly swollen, irregularly vacuolated with or without pyknotic nuclei (Fig. 30). In some spots the cells showed blue-stained sarcoplasm with karyorrhexis. There was an occasional mononuclear and neutrophilic infiltration. There were also foci of increased connective tissue in the upper portions.

Right bundle branch. In the first part of its course, the right bundle branch showed some degeneration equal to that of the

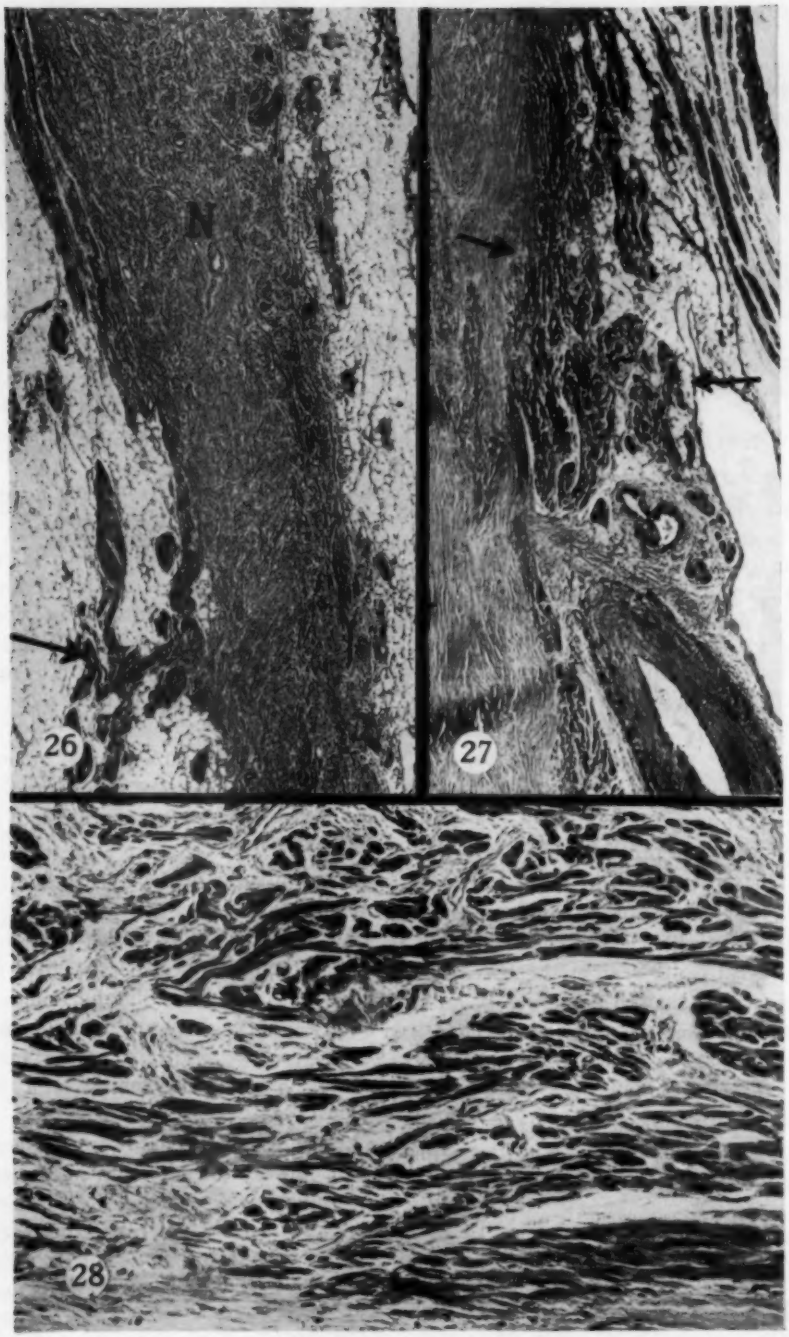


Fig. 26 (Case 3).—Topographic view of SA node and part of approaches, showing early necrosis of the approaches. Hematoxylin-eosin stain; × 30.

N, node. Arrow points to the approaches.

Fig. 27 (Case 3).—Topographic view of the AV node showing irregular staining of node and sclerosis of the ramus septi fibrosi. Hematoxylin-eosin stain, × 30.

Arrows point to the node.

Fig. 28 (Case 3).—Section of the AV node showing irregularity of staining. Hematoxylin-eosin stain; × 150.

Fig. 29 (Case 3).—Section of the AV bundle showing an increase in connective tissue, with focal degenerative changes. Hematoxylin-eosin stain; × 150.

Fig. 30 (Case 3).—Section of the left bundle branch showing severe degeneration and early necrosis. Hematoxylin-eosin stain; × 150.

bundle, which persisted up to about the muscle of Lancisi. For some distance there were no changes, but more distally small foci again appeared which became more intensive in its terminal portion. Here there was also a slight increase in connective tissue. This branch appeared to be less involved than the left branch.

Blood supply to the SA node. There was marked sclerosis with marked narrowing of

the right main coronary artery in the region of the origin of the ramus ostii cavae superiorae.

Blood supply to the AV node. There was marked narrowing of the right main coronary artery in the region of the ramus septi fibrosi. The direct branches to the node showed moderate thickening and narrowing.

PATHOLOGIC DIAGNOSIS

1. Coronary heart disease with marked narrowing of all major arteries and recent thrombi in the right circumflex and the anterior descending branches:
   (*a*) Recent, organizing, and old infarct of most of the left ventricle and ventricular septum, parts of the right ventricle, both atria, and the atrial septum

TABLE 4.—*Case 3—Clinicopathologic Correlation*

	Electrocardiographic Interpretation	Pathologic Changes in Conduction System
1	First- and second-degree AV block	1 Fibrosis of AV bundle and left and right bundle branches; acute degeneration of AV node and AV bundle
2	Complete left bundle branch block	2 Early necrosis of left bundle branch
3	.....	3 Acute degeneration of right bundle branch
4	.....	4 Fibrosis, small infarcts at approaches to SA node

   (*b*) Diminution of the blood supply to the SA and AV nodes, bundle, and bundle branches
   (*c*) Slight fibrosis of the AV bundle and right and left bundle branches
   (*d*) Acute degenerative changes in AV node, bundle, and right bundle branch
   (*e*) Early necrosis of the left bundle branch

CLINICOPATHOLOGICAL SUMMARY (TABLE 4)

Case 3 was a case of chronic coronary insufficiency created by narrowing of all the coronary arteries and of superimposed acute coronary insufficiency created by thrombi in the anterior descending and the right circumflex coronary artery in the region of its origin. This had resulted, on the one hand, in massive, acute organizing and old infarction of both ventricles, the ventricular septum, and to a smaller extent both atria and the atrial septum. On the other hand, it had narrowed or decreased the blood supply to the SA nodal region in part, and to a greater extent to the His-Tawara system. The ensuing damage to the approaches to the SA node, the atrial septum, the approaches to the AV node, the AV node itself, bundle, and bundle branches resulted in progressive degrees of heart block, from first- to second-degree AV block, and the development of complete left bundle branch block.

CASE 4.—*Clinical Summary.*—This 56-year-old white woman, a known diabetic of five years' duration, was admitted with a history of precordial distress of long duration, usually relieved by rest, and occasionally requiring glyceryl trinitrate. About three weeks prior to admission she began to experience easy fatigability, and two weeks before admission angina began to increase in frequency and severity.

Past history revealed that she had had a duodenal ulcer, and ten years previously, gall-bladder surgery.

Physical examination on admission showed the patient to be in no acute distress. There were moist rales in both bases. The blood pressure was 130/80, with regular sinus rhythm of 78. The point of maximal impulse was 2 cm. to the left of the midclavicular line. There was a Grade 3 apical systolic murmur transmitted to Erb's area. P_2 was greater than A_2. The liver was palpable one fingerbreadth below the costal margin and was smooth and nontender. Laboratory examination revealed a red blood cell count of 3,420,000 per cubic millimeter, with 11 gm. of hemoglobin per 100 cc. The white blood cell count was 11,150, with 60% neutrophiles, 29% lymphocytes, 4% monocytes, 2% eosinophiles, and 5% stab forms. There was 3+ sugar in the urine, with no acetone. The Kahn test was negative. X-ray examination showed a slightly enlarged heart, with prominence of the left ventricle and congestive changes in both hilar regions. Electrocardiographic examination will be discussed below.

Nineteen hours after admission the patient suddenly complained of protracted chest pain, went into shock, and died three hours later.

Electrocardiographic Data.—Nov. 9, 1951 (Fig. 31): First- and second-degree heart block. P-R interval, where first-degree heart block occurred, was 0.32 second with a conspicuous auricular T wave. P waves were broad and notched. Wenckebach phenomenon was present. QRS duration 0.14 second. Left bundle branch block, complete. Infarct of posterior wall and septum was suggested by ST changes. Ventricular premature systoles were present.

Electrocardiographic Interpretation.—In summary, the electrocardiograph showed damage to

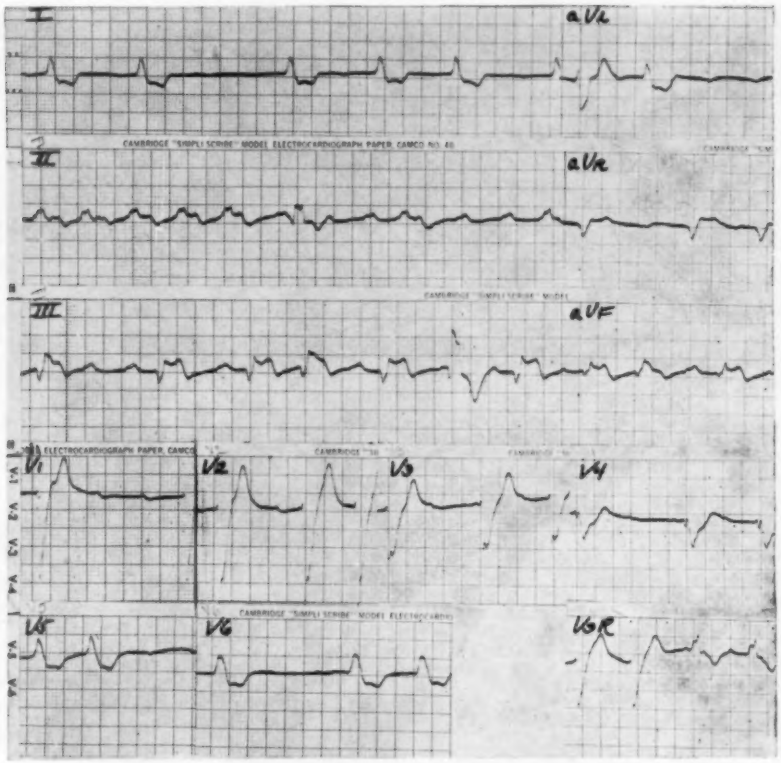


Fig. 31 (Case 4).—11/9/51. Sinus rhythm. Broad notched P waves with conspicuous auricular T waves. First- and second-degree AV block with Wenckebach phenomenon. QRS 0.14 second. Complete left bundle branch block. ST elevation in L_3, aV_F, V_{2R}, V_2-V_4 suggest septal infarct with anterior and posterior wall extension.

auricular muscle, the AV node and/or its approaches, the AV bundle, and the left bundle branch. The posterior wall of the left ventricle and the septum have been damaged. The presence of complete left bundle branch block made the exact localization of the infarct difficult to diagnose from the electrocardiographic viewpoint but indicated unquestionable massive septal infarction.

Clinical Diagnosis.—Arteriosclerotic heart disease with coronary thrombosis, with acute myocardial infarction. Diabetes mellitus.

POSTMORTEM FINDINGS

Apart from the findings in the heart, the pathologic diagnosis was: (1) arteriolar nephrosclerosis and (2) chronic, passive hyperemia of the lungs, with pulmonary edema.

Heart

Gross Examination.—The heart weighed 350 gm. The left ventricle was hypertrophied. The epicardium presented an area of red discoloration over the anterior and posterior walls. On section the left ventricular myocardium showed red areas in both the anterior and posterior walls. The lining of the coronary arteries presented numerous grayish-yellow plaques. These had narrowed the lumen of the anterior descending and left circumflex branches. The right coronary artery showed a recent thrombus near the acute margin of the heart. There were no further changes.

Microscopic Examination.—Pericardium: This showed focal infiltration of neutrophiles.

Endocardium and the Valves: These showed the usual aging changes.

Myocardium: Left ventricle. Both the anterior and posterior walls showed an acute, organizing and old infarct.

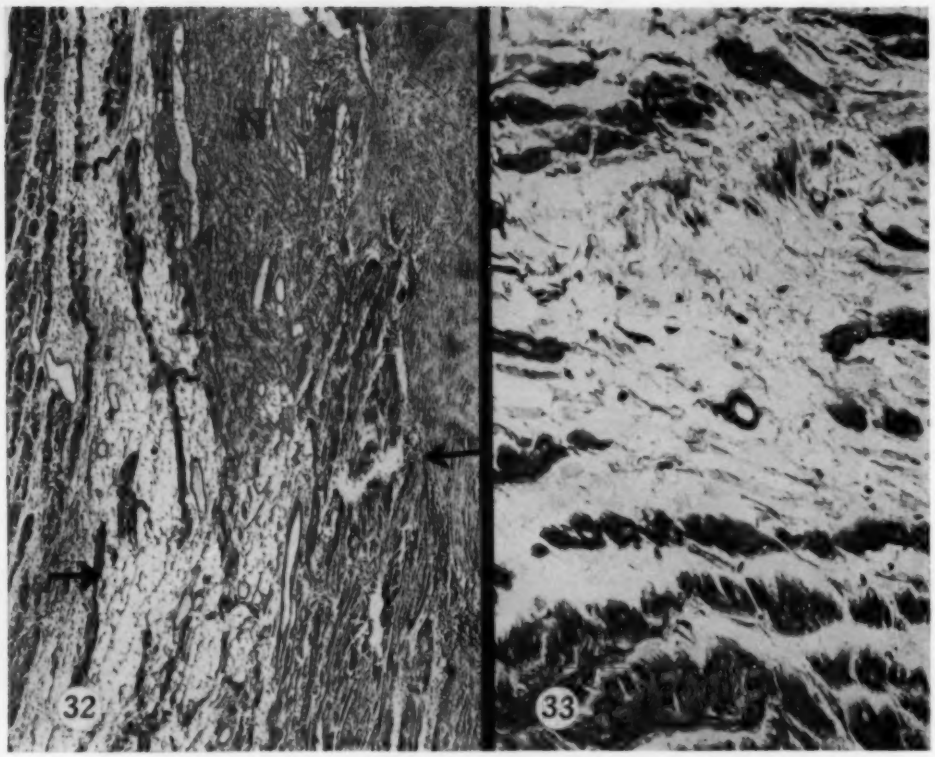


Fig. 32 (Case 4).—Topographic view of the AV node and its approaches, showing recent infarction of the approaches and the fringe of the node. Hematoxylin-eosin stain; × 30.

N, node. Arrows point to the approaches.

Fig. 33 (Case 4).—Section of AV bundle showing degeneration and dissolution of nodal tissue, with degeneration of collagen. Hematoxylin-eosin stain; × 150.

Right ventricle. The anterior wall showed a small, while the posterior showed a large, acute infarct.

Ventricular septum. This showed a recent organizing and old infarct of almost the entire septum.

Both atria and atrial septum. All of these showed areas of acute and organizing infarction.

Conduction System: SA node. There were no changes.

Approaches to the SA node. There was an occasional small scar in the atrial appendage, but the approaches were not impaired.

Approaches to the AV node. Considerable portions of the medial, inferior, and superior approaches were acutely infarcted (Fig. 32).

AV node. There was severe degeneration in the beginning of the node, with an infiltration of lymphoid cells. The distal part of the node presented no change.

AV bundle. The penetrating portion of the bundle presented no change. The branching portion, however, showed severe degenerative changes, becoming maximal at the bifurcation, with fraying and destruction of cells, and degeneration of the surrounding collagen (Fig. 33).

Left bundle branch. There was severe degeneration and early necrosis of this branch throughout its extent (Fig. 34).

Right bundle branch. There was severe degeneration and early necrosis at the beginning of the branch, which maintained its severity up to the muscle of Lancisi (Fig.

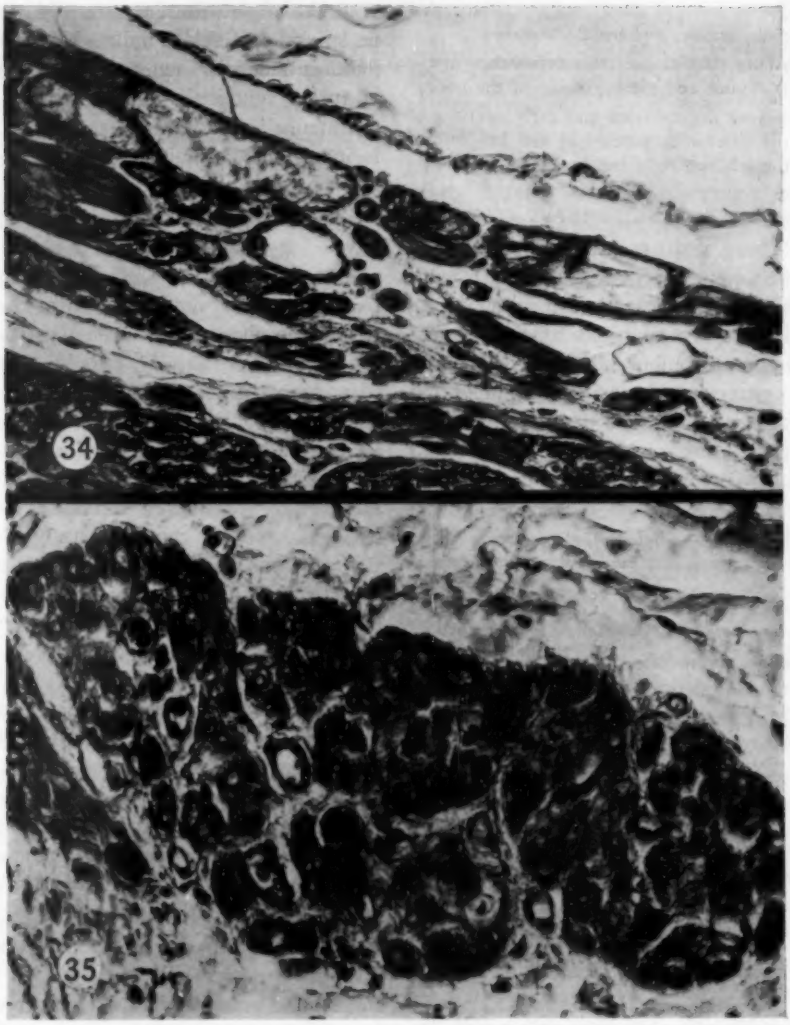


Fig. 34 (Case 4).—Section of the left bundle branch showing marked degeneration and early necrosis of fibers. Hematoxylin-eosin stain; × 150.

Fig. 35 (Case 4).—Section of the right bundle branch showing marked degeneration and early necrosis. Hematoxylin-eosin stain; × 300.

35). Distal to this point there were slight to moderate degenerative changes.

Blood supply to the SA node. There was moderate narrowing of the right main coronary artery in the vicinity of the ramus ostii cavae superiorae.

Blood supply to the AV node. There was narrowing of the right main coronary artery in the region of the ramus septi fibrosi.

PATHOLOGIC DIAGNOSIS

1. Coronary heart disease with narrowing of the left anterior descending, left circumflex, and right main coronary artery and recent thrombus in the right main artery at the acute margin:
   (*a*) Recent organizing and old infarct of the anterior and posterior walls of the left ventricle, the ventricular septum, with similar but less involvement of the right ventricle and both atria

(*b*) Impairment of blood supply to the AV node, bundle, and bundle branches

(*c*) Acute infarction of the approaches to the AV node and the beginning of the node

(*d*) Severe degeneration and early necrosis of the branching portion of the bundle and the left and right bundle branches

CLINICOPATHOLOGICAL SUMMARY (TABLE 5)

Case 4 was again a case of chronic coronary insufficiency produced by narrowing of all the coronary arteries, with superimposed acute coronary insufficiency produced by a thrombus in the right main coronary artery at the acute margin of the heart. This resulted, on the one hand, in massive, acute organizing and old infarction of the left ventricle and ventricular septum,

TABLE 5.—*Case 4—Clinicopathologic Correlation*

	Electrocardiographic Interpretation	Pathologic Changes in Conduction System
1	First- and second-degree AV block with Wenckebach phenomenon	1 (*a*) Acute infarct at approaches to AV node and beginning of AV node; (*b*) early necrosis of branching portion AV bundle
2	Complete left bundle branch block	2 Early necrosis of left bundle branch
3	.....	3 Early necrosis of right bundle branch

and to a slighter extent the right ventricle, both atria, and the atrial septum. On the other hand, it had narrowed or diminished the blood supply to the AV node, bundle, and bundle branches. The ensuing damage to the atrial septum, to the approaches of the AV node, the AV node itself, bundle, and bundle branches resulted in first- and second-degree AV block, as previously, with the addition in this case of a Wenckebach phenomenon almost approaching complete heart block. In addition, complete left bundle branch block had occurred.

GENERAL COMMENT

As intimated above, if the prevailing theory of conduction in the human heart is accurate, then a proper pathologic study of conduction must of necessity include the following structures: (1) the SA node, (2) the approaches to the SA and AV nodes, (3) the atrial musculature, (4) the AV node, (5) the AV bundle (His), the right and left bundle branches to their termination, and the ventricular myocardium. It may eventually be determined that such a study is incomplete without a study of the nerve supply to the above structures.

Such a study as outlined must of necessity be at least semiquantitative. It is self-evident that isolated sections through these structures may not necessarily reveal the pathologic change or, if seen, could not be evaluated quantitatively. It is for that reason that both Mönckeberg and Mahaim felt compelled to use serial sections in their study of the AV node, bundle, and bundle branches. Such studies necessitate 4 to 6000 sections for the latter structures alone in a single heart. A complete study of all the structures as we have outlined would give an astronomical number of sections and permit the technician to section only one heart a year.

It is for that reason that one of us (M. L.) evolved methods for study which, we think, satisfy the following criteria: (1) They permit the complete visualization of all the structures in continuity, and (2) they permit a semiquantitation of the changes noted. It has been previously shown that these methods permit proper visualization of all structures. The second criterion was tested in the present work by doing serial sections through the SA and AV nodes, bundle, and bundle branches in the first three cases. In studying the pathology, approximately every 40th section was looked at and the findings recorded, and then several alternate sets of every 40th section were studied. The findings of these preliminary studies were then compared with the complete study of all the sections. Very little, if any, difference in findings was found. We hope to repeat this maneuver in many more cases, but it would seem that the abbreviated method will prove to have validity.

The pathologic changes found in the AV node, bundle, and bundle branches have been repeatedly reviewed. These can be grouped

under (1) inflammatory, (2) degenerative and necrotic, (3) neoplastic, and (4) abnormality in development. From the etiological standpoint changes in the conduction system may be found in (1) acute and subacute endocarditis, (2) myocarditis, (3) pericarditis, (4) coronary ischemia, (5) primary or metastatic tumors, and (6) congenital heart disease. In endocarditis there may be spread of inflammation from the mitral and aortic valves to the AV node and bundle, and sometimes to the beginning of the left bundle branch. Rarely is the tricuspid valve the source of such pathologic change. In pericarditis the SA and AV nodes and the AV bundle may be especially involved. In myocarditis all parts of the system may be involved. In the latter disease there may be marked involvement of the myocardium with minimal change in the conduction system or maximal involvement of the conduction system with relatively little change in the myocardium.

In coronary ischemia Mahaim has already delineated several types of involvement of the AV node, bundle, and bundle branches, based on their blood supply. The blood supply to the AV node and bundle, and to a varying extent the beginning of the bundle branches, comes from the ramus septi fibrosi, rarely augmented by branches from the anterior descending coronary artery. The ramus septi fibrosi originates from the main stem of the right coronary artery before it forms the posterior descending, in about 90% of the cases, and in the remainder of the cases, from the left coronary artery when it forms the posterior descending. The blood supply of the first part of the right bundle branch comes from the ramus septi fibrosi, that of the first or first two anterior perforating arteries from the anterior descending, and perhaps the ramus cristae supraventricularis, and that of the second posterior perforating comes from the posterior descending. That of the second part comes from the ramus limbi dextri, which is a branch of the second anterior perforating, reinforced by the anterior and, questionably, the posterior perforating arteries. The third part comes from the ramus limbi dextri anastomosing with branches from the right coronary artery feeding the myocardium. The blood supply of the anterior radiation of the left bundle branch comes from the anterior perforating branches of the anterior descending, and that of the posterior radiation, from the posterior perforating branches of the posterior descending. It is thus evident that the blood supply of the AV node and bundle is most frequently from the right coronary artery, the beginning of the right and left bundle branches from the right and left coronary arteries, the middle part of the right branch mostly from the left, the terminal portion of the right from both, and the left branch distal to its origin from both the right and left.§

In the high right coronary type of Mahaim there is obstruction in the right coronary above the origin of the ramus septi fibrosi. Here the ischemia may involve the AV node and bundle with or without the beginning of the left and right bundle branches. This may be associated with a posteroseptal infarct accompanied by infarction of the atria. In the low right coronary type there is obstruction of the posterior descending coronary (below the origin of the ramus septi fibrosi). This may result in a variable ischemia to the posterior radiation of the left bundle branch and may be associated with varying signs of a posteroseptal infarct with varying conduction disturbances of the left bundle branch. In the left type there is obstruction to the first portion of the left coronary artery. This may result in ischemia of most of the right bundle branch, with right bundle branch block associated with an anteroseptal infarct.

To these types we may add a fourth, the complete right, where the obstruction is at the beginning of the right coronary artery, before the origin of the ramus ostii cavae superiorae. This vessel supplies the SA node and originates from 1 to 3 cm. from the origin of the right coronary artery. Occasionally it is a branch of the left. When the

§ References 2, 10, and 11.

right coronary is obstructed at this point, there may be ischemia of the SA and AV nodes and AV bundle, associated with infarction of both atria, the posteroseptal walls of both ventricles, and the anterior wall of the right ventricle. As in the remainder of the myocardium, gradual occlusion of one vessel may not necessarily produce ischemia of the conduction system, because of the ample anastomoses.

Our Cases 1 and 2 show the usual effects of acute and chronic myocarditis on the conduction system. In addition, in Case 1 the importance of the study of the approaches to the AV node is clearly seen, since this shares with the beginning of the AV node in the genesis of the block. It also brings out the importance of the ventricular myocardium in the prognosis of block. Where idioventricular rhythm is temporarily or permanently established, the ultimate outcome will depend upon the integrity of the myocardium. The partial left bundle branch block in Case 1 was found to have a morphologic base. In Case 2 the presence of periods of nodal rhythm is explained by the irritability of the AV node. It is to be noted that this structure, although inflamed, was not necrotic. Mahaim has pointed out the great propensity for irritability of the conduction system in inflammatory states. The presence of right bundle branch block in the early course of Case 2 points to the well-known vulnerability of the right branch to contiguous septal change. Whereas the left branch fans out into two widespread radiations, the right branch remains a solitary trunk and hence vulnerable to localized destruction.

Cases 3 and 4 are examples of ischemia of the conduction system brought about by severe involvement of both major coronary arteries, with massive infarction of the ventricles and, to a less extent, the atria. In both, there is evidence of varying degrees of AV block associated with complete left bundle branch block. This, as pointed out by Mahaim, can be attained only by severe involvement of both major coronary arteries and hence imparts a serious prognosis—for this, of necessity, must involve the myocardium massively. Likewise, the presence of electrocardiographic evidence of atrial pathology in ischemic disease portends a serious outlook.

In Case 3 the involvement of the right coronary is complete, while in Case 4 it is high. There are thus considerable changes in the SA node region in Case 3 which are minimal in Case 4, and severer changes in the anterior wall of the right ventricle in Case 3 as compared with Case 4. From the electrocardiographic standpoint, however, this difference cannot be discerned from presently known criteria. However, the presence of a significant *q* wave in the left precordial leads in Case 3 (absent in Case 4) in the presence of complete left bundle branch block may suggest serious damage to the right ventricle. This awaits further clinicopathologic correlation in a large series of cases.

It may be further noted in Case 3 that extensive changes may be present in the approaches to the SA node, with persistence of sinus rhythm. This corresponds to the well-known difficulty in ablating the node experimentally.

Finally, the observation of Mahaim is here confirmed that pathologic changes in the conduction system are usually not single, but multiple, and thus a large series of cases must be studied for finer electrocardiographic and pathologic correlation.

SUMMARY

A histopathologic study of the conduction system and of the remainder of the heart was made in four cases which presented clinical and electrocardiographic evidence of severe or complete heart block. Case 1 was the seat of a chronic organizing myocarditis, Case 2 of an acute fulminating myocarditis, and Cases 3 and 4 of severe acute and chronic coronary ischemia.

The electrocardiographic findings were found to correlate well with the changes in the conduction system.

Pathologic changes in the conduction system are usually multiple, as previously pointed out by Mahaim.

The findings of partial heart block with complete left bundle branch block is of serious import, as previously pointed out by Mahaim.

REFERENCES

1. Mönckeberg, J. G.: Untersuchungen über das Atrioventrikularbündel im menschlichen Herzen, Jena, Gustav Fischer, 1908.

2. Mahaim, I.: Les Maladies organiques du faisceau de His-Tawara: Les Syndromes coronaires; L'Endocardite septale; L'Infarctus septal, Paris, Masson & Cie, 1931.

3. Gross, L., and Fried, B. M.: Lesions in the Auriculoventricular Conduction System Occurring in Rheumatic Fever, Am. J. Path. **12**:31, 1936.

4. Rosenthal, S. R.: Branch Arborization and Complete Heart Block, Arch. Int. Med. **50**:730, 1932.

5. Lev, M.; Widran, J., and Erickson, E. E.: Method for the Histopathologic Study of the Atrioventricular Node, Bundle, and Branches, A. M. A. Arch. Path. **52**:73, 1951.

6. Erickson, E. E., and Lev, M.: Aging Changes in the Human Atrioventricular Node, Bundle, and Bundle Branches, J. Gerontol. **7**:1, 1952.

7. Lev, M., and Watne, A. L.: Method for Routine Histopathologic Study of Human Sinoatrial Node, A. M. A. Arch. Path. **57**:168, 1954.

8. Lev, M.: Aging Changes in the Human Sinoatrial Node, J. Gerontol. **9**:1, 1954.

9. Lev, M.: Is There a Specialized Muscular Conduction System in the Mammalian Heart? Am. Pract. **4**:92, 1949.

10. Gross, L.: Blood Supply to the Heart in Its Anatomical and Clinical Aspects, New York, Paul B. Hoeber, Inc., 1921.

11. Lascano, E. F.: Irrigación normal del nódulo de Tawara, haz de His y sus ramas, Rev. argent. cardiol. **10**:23, 1943.

Recurrent Carotid Body Tumor

Report of a Case

VICTOR M. AREAN, M.D., San Juan, Puerto Rico

The carotid body was described by von Haller in 1743, but the tumor arising in that structure was first recorded almost 150 years later by Marchand (1891).* In 1941, Harrington and co-workers [1] published their findings on 19 cases seen at The Mayo Clinic. In 1951, Lahey and Warren [2] reported the largest individual series thus far published. Morfit and collaborators,[3] in a review of the literature extending to 1952, were able to collect 275 cases, adding 12 of their own. The total to date is probably not more than 300 cases.

The first surgical excision of a carotid body tumor was performed by Madyl in 1866, with successful recovery. Albert was the first, in 1899, to excise such a tumor without sacrificing the carotid artery, but the mass recurred within a year after the operation.

Much controversy has existed in regard to the incidence of malignant manifestations in this type of neoplasm, and, although the number of cases reported with generalized metastasis is appreciable, definite proof of such an occurrence is lacking in the great majority of cases. Harrington and co-workers [1] estimated that 50% of their cases were malignant growths, but they followed purely histologic criteria; in only two of their cases did the growths recur locally. One of these patients died of what was assumed to be cerebral metastasis, but this was never proved histologically. In 1947, Lahey and Warren,[4] citing Gratiot, stated that the incidence of malignant manifestations ranged from 15% to 20% of cases. Yet, a reevaluation of their own series in 1951 [2] showed that none could be considered malignant growths, and they questioned the validity of such an interpretation in many instances. The view of these authors is that these tumors are rarely malignant.

Willis [6] maintained that, except for a few doubtful cases, most carotid body tumors are benign and slow-growing. LeCompte † in an extensive and thorough study of these neoplasms was unable to convince himself of the malignant nature of the cases reported as such, except that of Pendergrass and Kirsh.[9] Since the publication of his monograph on the subject, two more cases have been added to the literature, one by Romanski [10] and the other by Morfit and collaborators,[3] both cases showing proof of generalized metastasis.

Among the 300 cases thus far reported, only 11 growths recurred locally after excision. When such recurrence took place, the tumor was found to have invaded the surrounding tissues, making total resection hazardous and sometimes impossible.

Lahey and Warren [2] and LeCompte ‡ have warned against radical surgery in tumors of the carotid body and advise only a confirmatory and diagnostic biopsy. These authors feel that since most of these neoplasms are benign, and since radical surgery is attended with a mortality of 30%, the latter treatment is not indicated. Neither Matas' test nor any other procedure that may help in the development of the collateral circulation to the brain, such as Dandy's operation, is reliable in avoiding postoperative complications. Since these tumors are usually intimately adherent to the carotid artery, it is not always possible to resect them without damaging the vessel,

Received for publication July 14, 1955.

Associate Professor of Pathology, School of Medicine and School of Tropical Medicine, of the University of Puerto Rico.

* Marchand,[5] cited by Lahey and Warren.[4]

† References 7 and 8.

‡ References 7 and 8.

even when the latter is not ligated. The usual result is an ascending thrombosis with the inevitable sequela of permanent hemiplegia or of death subsequent to cerebral infarction. On the other hand, when to avoid damage to the artery the tumor is only partly excised, recurrence will certainly take place. Operation on recurrent tumors is usually difficult and frequently incomplete, and the incidence of complications, particularly cerebral, is greater than that following primary operations.

The following case is reported because of the relatively low incidence of these tumors, the infrequency of recurrence, and the unusual histologic behavior of this neoplasm.

REPORT OF CASE

A 66-year-old mulatto man was admitted to the San Juan City Hospital on Feb. 3, 1948, because of a mass on the left side of the neck. Two years before admission, following a fall, he had noted a small swelling in that area. This grew slowly until about four months before admission, when it began to increase in size rapidly. There had been no pain or tenderness and no difficulty on swallowing. Physical examination revealed a mass the size of an orange located on the left side of the neck. It was soft, painless, and immovable and extended from the angle of the jaw to the clavicle. The rest of the examination was negative, except for bilateral hydroceles. The tumor was excised with the patient under general ether anesthesia. The surgeon thought he was dealing with a so-called "aberrant thyroid" and noted that the tumor was friable and bled easily. The trachea and larynx were displaced to the right. No details concerning its relation to the carotid artery and other local structures were given. The patient did well postoperatively. He failed to report for follow-up and was not seen until two years later.

At that time, a mass measuring about 8 cm. across was located at the site of the previous operation. It was tender, painful, lobulated, and sharply defined. At operation it was felt that the tumor mass had invaded the surrounding muscles, eroding through the cervical spine at the level of C_4-C_5. After the carotid artery, the vagus nerve, and the sympathetic chain had been dissected away from the mass, the latter was resected. The tumor encroached so closely upon the phrenic nerve that it had to be ablated. The surgeon could not remove the interspinal and intraspinal extensions of the neoplasm. The patient recovered rapidly and was discharged seven days after the operation.

Gross Examination

The specimen consisted of an irregular, roughly spherical portion of firm tissue with dark red-brown mottling and measured 7.5×6.5×3.4 cm. The surface was covered with fibrofatty tags and was moderately lobulated. The cut surface was light tan, homogeneous, and dense.

Microscopic Examination

The tumor was composed of groups of polyhedral cells separated from one another by bands of connective tissue (Fig. 1). The cells had a round or ovoid nucleus, with finely granular chromatin and sharply defined nuclear membrane. The cytoplasm was clear and slightly granular, although the granulations were poorly defined. The cell borders also were ill-defined. Silver impregnation stains revealed two types of reticulum fibers; one was broad, deeply stained, and surrounding large groups of cells; the others were fine, short fibrils wrapped around individual cells or surrounding groups of them (Fig. 2). There was abundant vascularization, with vessels varying greatly in diameter (Fig. 3). The endothelial cells were inconspicuous. Some fibroblasts were interspersed among the tumor cells. Pleomorphism, giant cells, and mitotic figures were not visible. In some areas the tumor was separated into

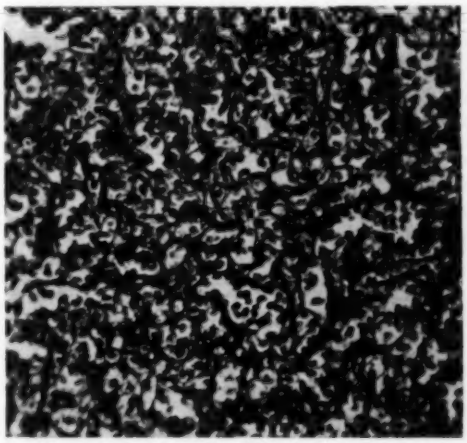


Fig. 1.—Arrangement of tumor cells in nests separated from connective tissue fibers (tumor removed at second operation). Wilder's reticulum stain; reduced about ⅙ from mag. × 380.

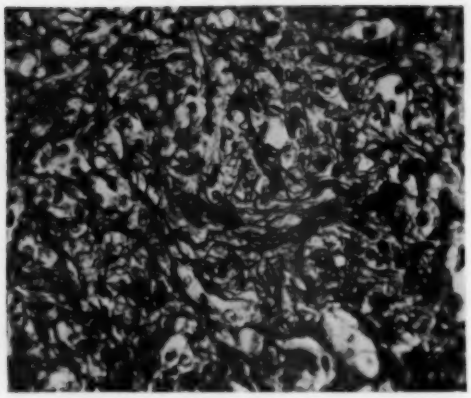


Fig. 2.—Pattern of reticulum demonstrating thin and coarse fibers. Wilder's reticulum stain; reduced about ⅓ from mag. × 380.

lobules by broad bands of collagenized connective tissue. Foci of old and recent hemorrhage could be seen in the connective tissue. Review of the tissues removed at the first operation showed essentially the same appearance.

Subsequent Course

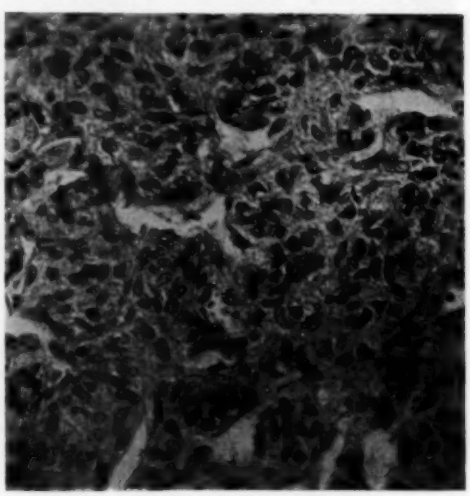
The patient returned on Dec. 12, 1953, complaining of tenderness and swelling at the site of operation. Physical examination disclosed a large, hard, tender mass extending from the mastoid process alongside the lower edge of the mandible to the anterior aspect of the neck. He was again operated upon, a mass measuring 10 cm. in diameter being removed. It was intimately adherent to the carotid sheath, from which it had to be separated by sharp dissection, after which the wall of the carotid artery was noted to be very thin. The tumor had also extended into the spinal canal, so that a radical resection could not be attempted. During the operation, which was laborious because of adhesions and excessive bleeding, a ligation of the anterior vertebral artery was performed. The operation went on for five hours, and there were moments during which the pulse and blood pressure were unobtainable. The patient left the operating room in critical condition and stayed in the recovery room for two days. He was given blood transfusions, digitalis, and oxygen. Shortly after recovery from anesthesia right-sided hemiplegia was noted. Two days later edema and tiny blebs were observed in the right upper arm. During the following days the patient seemed to be recovering nicely, except for the hemiplegia. On the seventh postoperative day he developed rales and dyspnea, and within a few hours he became stuporous, dying quietly soon thereafter.

Autopsy Report

The main findings were confined to the site of operation, the brain, and the lungs. At the operative site there was a mass of grayish-white firm tissue, ill-defined and covering an area approximately 5 cm. broad. The tumor tissue penetrated through the intervertebral space and eroded the fourth and fifth cervical vertebrae, causing moderate displacement but no compression of the spinal cord. It was not attached to the meninges. The common carotid artery was occluded by a thrombus that extended from about 2 cm. below the bifurcation to the base of the skull along the internal carotid artery. Grossly the brain was edematous. The nucleus pallidus and striatum, on the left side, showed an area of hemorrhage measuring 2 × 1 cm. and extending for a distance of 2 cm. into the hypothalamic region. In the left occipital lobe there was another hemorrhage measuring 3.5 × 4.5 cm. The cerebellum was normal.[1] Grossly, the lungs were heavy and congested, with numerous small, grayish, and firm foci. Blood-tinged fluid exuded from the cut ends of the bronchi.[2] Microscopically, the brain showed multiple foci of infarction, some several days old and others more recent. Fresh perivascular hemorrhages were seen throughout the brain substance. The lungs showed congestion and scattered foci of bronchopneumonia bilaterally.

The specimen of tumor removed at operation measured 10 cm. in largest diameter. It was firm, pinkish-gray, and resilient. Microscopically, it showed many regions with features identical with those described in the for-

Fig. 3.—Abundant blood vessels of different caliber ("angiomatoid" pattern). Masson-Goldner trichrome stain; reduced about ⅓ from mag. × 380.



mer specimens. However, there were many areas where cellular pleomorphism was more striking (Fig. 4). The latter areas were composed of small cells with a round or ovoid nucleus, finely divided chromatin, and a sharply delimited membrane. The cytoplasm was scanty. Other cells showed large, pleomorphic nuclei, although none was gigantic. Multinucleated cells and mitoses were not seen. The tumor was much more cellular than in previous examinations, and the previous arrangement into groups of cells was mostly replaced by a diffuse growth. In some regions the tumor had a marked tendency to collagenization, well demonstrated with the Masson-Goldner trichrome stain. Foci of lymphocytic infiltration were also seen. There was no histologic evidence of invasion of surrounding tissues, blood vessels, or lymph nodes. Sections from the fourth and fifth vertebrae were free of tumor.

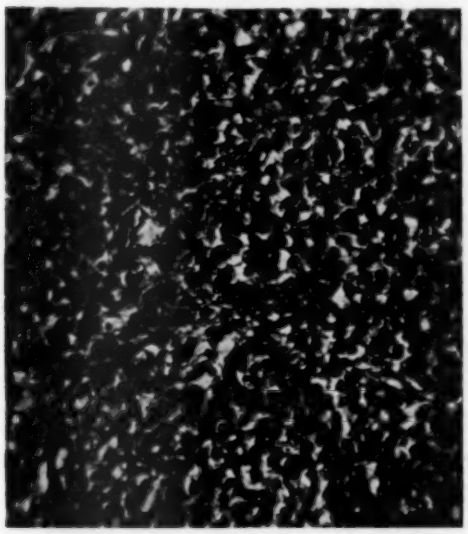


Fig. 4.—Increased cellularity, marked pleomorphism, and haphazard arrangement of tumor cells (tumor obtained at autopsy). Wilder reticulum stain; reduced about ⅙ from mag. × 380.

COMMENT

The first reported case of carotid body tumor recurring after surgical removal was that of Albert in 1899, following which 11 cases have been published. In the present case the tumor appeared seven years before death and recurred three times after surgical interventions, the intervals between the recurrences becoming increasingly shorter. Although the growth appeared fairly well differentiated histologically at the beginning, as the rate of growth increased its pleomorphism and cellularity became more marked, until finally only a few areas showed a suggestion of its true nature. The reticulum pattern was not too characteristic and became more irregular as the tumor became more undifferentiated. On the whole, the tumor adopted a pattern highly suggestive of malignant transformation. However, there is not enough histologic or clinical evidence to support this diagnosis. It is true that the tumor recurred repeatedly and eventually caused the death of the patient, but the latter was the result of a complication rather than of the tumor itself. While it has been considered that pleomorphism, giant tumor cells, and invasion of capsule or blood vessels are indicative of malignant transformation, LeCompte [7] denies such criteria as holding true for this type of neoplasm, and I agree with him. Foot, in a personal communication to Morfit,[3] also doubted the advisability of making a diagnosis of malignant transformation in carotid body tumors purely on histologic bases.

In the present case the tumor had extended into the spinal canal, causing, however, no alteration of the cord and not invading the vertebrae. Despite the prolonged course and repeated recurrences, no evidence of visceral, lymph node, or bone metastasis was found at autopsy.

SUMMARY

A case of recurrent carotid body tumor is recorded. The tumor was present over a period of seven years, during which time the patient underwent several operations followed by three recurrences. The intervals between the latter became shorter as time passed. Likewise the histologic appearance, which at first was well differentiated, became more cellular and pleomorphic until at autopsy only a few areas showed a suggestion of the tumor's original nature.

Despite the long survival period and the recurrences, there was no evidence of metastatic spread, of invasion of surrounding tissues, or of any of the features generally accepted as indicative of malignant transformation. The need for caution as to radical surgery for this type of neoplasm is discussed because of the high postoperative mortality resulting from thrombosis of the homolateral carotid artery.

Dr. Enrique Koppisch and Dr. Philip LeCompte gave valuable suggestions, and Mr. Antonio Puras did the photomicrographic work.

REFERENCES

1. Harrington, S. W.; Clagett, O. T., and Dockerty, M. B.: Tumors of the Carotid Body. Clinical and Pathological Considerations of Twenty Tumors Affecting Nineteen Patients (One Bilateral), Ann. Surg. **114**:820-833, 1941.

2. Lahey, F. H., and Warren, K. W.: Long Term Appraisal of Carotid Body Tumors with Remarks on Their Removal, Surg. Gynec. & Obst. **92**:481-491, 1951.

3. Morfit, H. M.; Swan, H., and Taylor, E. R.: Carotid Body Tumors: Report of Twelve Cases, Including One Case with Proved Visceral Dissemination, A. M. A. Arch. Surg. **67**:194-214, 1953.

4. Lahey, F. H., and Warren, K. W.: Tumors of the Carotid Body, Surg. Gynec. & Obst. **85**:281-288, 1947.

5. Marchand, F.: Beiträge zur Kenntniss der normalen und pathologischen Anatomie der Glandula carotica und der Nebennieren, Internat. Beitr. wissensch. Med. Festschrift. Virchow **1**:535-581, 1891.

6. Willis, R. A.: Pathology of Tumours, St. Louis, The C. V. Mosby Company, 1948, pp. 873-875.

7. LeCompte, P. M.: Tumors of the Carotid Body, Am. J. Path. **24**:305-321, 1948.

8. LeCompte, P. M.: Tumors of the Carotid Body and Related Structures (Chemoreceptor System), in Atlas of Tumor Pathology, Washington, D. C., Armed Forces Institute of Pathology, 1951, Section IV, Fascicle 16.

9. Pendergrass, E. P., and Kirsh, D.: Roentgen Manifestations in the Skull of Metastatic Carotid Body Tumor (Paraganglioma), of Meningioma and of Mucocele: Report of 3 Unusual Cases, Am. J. Roentgenol. **57**:417-428, 1947.

10. Romanski, R.: Chemodectoma (Non-Chromaffinic Paraganglioma) of the Carotid Body with Distant Metastases, Am. J. Path. **30**:1-13, 1954.

News and Comment

ANNOUNCEMENTS

A. M. A. Annual Meeting, Chicago, June 11-15, 1956.—The 105th Annual Meeting of the American Medical Association will be held in Chicago, June 11-15, 1956, with a full program of lectures, television, motion pictures, and scientific exhibits.

The lecture program for each Section is prepared by the Section Secretary, and prospective participants should communicate as soon as possible with the Secretary of the Section before which they hope to read a paper. Application blanks for space in the Scientific Exhibit are now available and may be obtained from the Section Representative to the Scientific Exhibit.

Section on Pathology and Physiology

Secretary: Edwin F. Hirsch, M.D., 1439 S. Michigan Ave., Chicago 5.

Exhibit Representative: Frank B. Queen, M.D., 3181 S.W. Sam Jackson Park Rd., Portland 1, Ore.

Additional information may be obtained from the Secretary, Council on Scientific Assembly, American Medical Association, 535 N. Dearborn St., Chicago 10.

Mycosis Fungoides Followed for Fourteen Years

The Case of Dr. W. B. Cannon

JOSEPH C. AUB, M.D., Boston
S. BURT WOLBACH, M.D.†
B. J. KENNEDY, M.D., Minneapolis
and
ORVILLE T. BAILEY, M.D., Indianapolis

In 1932 a biopsy specimen of the skin of Dr. Walter B. Cannon, the great physiologist, presented evidence of mycosis fungoides. With the objectivity so well known to his friends and associates, he suggested that repeated biopsies be made whenever they would provide information on the development of this incompletely understood condition. The material thus obtained over a period of 14 years and that from the necropsy were carefully prepared for histologic study. They now afford an opportunity to trace the sequences involved in mycosis fungoides with development of lymphatic leukemia, to elucidate some controversial points concerning the cellular components involved, to review the hematologic data, and to consider the relation of the condition to exposure to unfiltered x-radiation. Therefore, the record demands careful reporting, and it is coupled with Dr. Cannon's name because a great man who taught so many students so much should also be associated with the record of his instructive illness.

Received for publication Aug. 20, 1955.

This is publication No. 853 of the Cancer Commission of Harvard University.

From the Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital, and the Pathology Department of The Peter Bent Brigham Hospital, Boston.

† Dr. Wolbach died March 19, 1954.

From a clinical point of view, the course of the disease is not exceptional; the value of this case lies in the careful evaluation of its pathologic and hematologic manifestations. The prolonged duration and development of leukemia are not common, but they occur often enough to be well recorded by Gall and Mallory,[1] by Warthin,[2] and by others.

Lever [3] has summarized the known pathologic characteristics of mycosis fungoides in his interesting book. Certainly the disease is closely related to the lymphomas, and involvement of internal organs may be encountered. At the Massachusetts General Hospital, Dr. Lever finds visceral lesions in not more than 20% of the necropsies on mycosis fungoides patients, and usually the visceral lesions are not prominent or widespread.*

None of the cases of Cawley and coworkers [4] showed blood changes, but 8 of their 10 patients studied at necropsy showed visceral involvement. These authors reached the conclusion that mycosis fungoides should be classified among the lymphoblastomas and upheld its "alleged reticulo-endothelial origin." One of their patients lived 14 years; the average duration of life was 5 3/10 years after the diagnosis was established. Heite and Socha [5] reached similar conclusions and found a high incidence of visceral lesions.

CLINICAL STUDY

Dr. Cannon was a pioneer in the use of the x-ray in physiologic experimentation. From 1897, when he was a first-year medical student, through 1908, he studied the move-

* Lever, W. F.: Personal communication to the authors.

ments of the gastrointestinal tract in animals by means of a primitive x-ray tube. An insatiable worker, his exposure to the x-radiations must have been great, as he hovered over the fluoroscopic screen above the x-ray table. He was saved from greater exposure by an astute guess of Prof. Henry P. Bowditch, who thought this new apparatus might be dangerous. They soon came to the conclusion that Dr. Cannon experienced acute x-ray burns because of electrostatic activity of the brushes in the motors. Therefore, very early in his experimental work, Dr. Cannon placed a galvanized iron box around his apparatus and attached a wire to the steam radiator to ground the charge and to diminish the danger of sparking. The box completely surrounded the x-ray apparatus except for a small opening at the top. As a result, he was relatively well protected from x-rays except for his hands, which were seriously burned repeatedly, and, to a less extent, his face and the upper part of his body. In 1908 Dr. Cannon ceased using x-rays for his investigations and went on to other fields of research.

Twenty-three years after Dr. Cannon had stopped exposure to x-ray there appeared itching of the skin and fiery red papular cutaneous lesions on his back, chest, thighs, knees, and elbows. This occurred in the summer of 1931, when he was 59 years old. During the next year these lesions and the pruritus were aggravated by attacks of indigestion, and exacerbation of the skin disease was ascribed to the ingestion of specific foods or to contact with laboratory animals.

Before that time there had been no serious illness other than pyelitis at the age of 54, followed by a chronic asymptomatic bacilluria. A papilloma of the bladder had been removed several months before the onset of the skin disease.

While Dr. Cannon was leading an active outdoor life during the summer of 1932, the itching subsided considerably. Nevertheless, it flared up after constipation, and the skin remained red with gray patches on the forearm. Biopsies of these lesions in the fall of 1932 showed mycosis fungoides.

In October, 1933, the skin involved was irradiated weekly with small amounts of low voltage x-ray to give temporary relief from the severe and progressive itching. Dr. Cannon was a man who expected continuous investigation, with the result that high- and low-carbohydrate, fat, and protein diets, as well as a ketogenic diet, were instituted one after another. He came to the hospital for his meals, and the diets were rigidly adhered to, without demonstrable improvement. Various vitamins were next tried in high concentrations—the B group and vitamin C. Concomitant with the continued x-ray treatments, 80 drops (about 4.8 cc.) of halibut liver oil with viosterol (50,000 units of vitamin A and 10,000 units of vitamin D per gram) was administered daily, and the itching promptly subsided. This was associated with a smoother appearance of the skin. Since the remission was preceded by repeated x-ray therapy, any possible benefit from the viosterol could not be evaluated adequately, though prompt relief was obtained, whereas previous efforts during x-ray therapy had failed. X-ray treatments were now stopped, and they were not found necessary again for many years. During the next two years vitamin therapy was continued. At the end of that period viosterol administration was discontinued and the itching promptly returned. The skin was softer and less red, but a skin biopsy in November, 1935, revealed changes similar to those originally noted three years earlier.

During the ensuing three years the skin of the arms, legs, and face became drier and desquamated. Since vitamin D in highly purified form proved to be less satisfactory than fish oil, the administration of halibut liver oil with viosterol was continued. A commercial preparation containing vitamin D (Navitol), 75 drops (about 4.5 cc.) daily, was given, commencing in October, 1938. Though Dr. Cannon was convinced of its beneficial effect, use of this therapy in other similar cases has not been dramatic; however, it sometimes appears to diminish the intense itching of the disease.

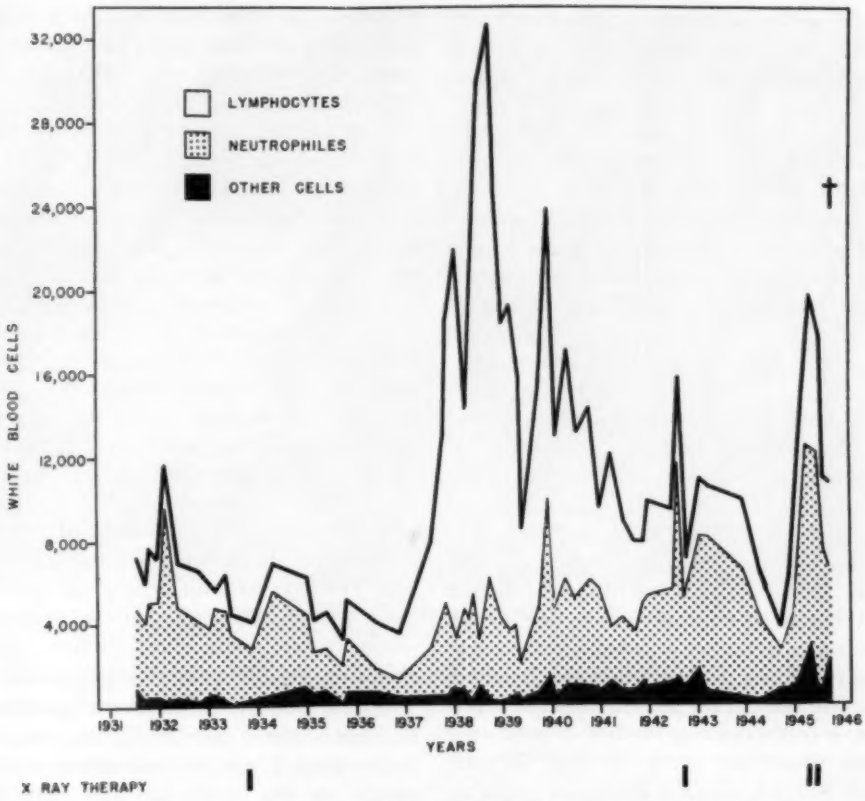


Fig. 1.—Variations in white blood cell counts, 1931-1945. Note the development of a picture of lymphatic leukemia beginning in 1937 and its gradual subsidence. The peaks in polymorphonuclear leucocytes were related to intercurrent infections.

During 1937 a progressive rise in the white blood cell count occurred, reaching a peak of 32,700 per cu. mm. in September, 1938, without evidence of infection. The differential count revealed 81% lymphocytes, 18% polymorphonuclear cells, and 1% monocytes. The lymphocytes were small, with very little cytoplasm, and the nuclei often were lobulated. A progressive improvement of the skin was demonstrated during the succeeding two years, but redness and heavy desquamation persisted. The elevation of the white blood cell count with predominance of lymphocytes was present until it slowly subsided to normal in 1941 (Fig. 1).

As a consequence of the exposure to x-rays in Dr. Cannon's experimental work between 1897 and 1908, the skin of the hands became dry and scaly and a large horny ulcerated lesion developed on the left wrist. This was excised in March, 1940, and proved to be a slowly growing epidermoid carcinoma. Other benign keratotic lesions were removed in 1941, and a basal-cell carcinoma beneath the right nares was treated with 25 mg.-hr. of radium at the same time. The skin, with the exception of that of the hands, remained smooth and pale, and there was only minimal itching.

An unusual manifestation of mycosis fungoides appeared in 1942. On Sept. 5, a sense of fulness was noted in the right ear, and an earache appeared two days later. Loss of the sense of taste for sweets on the right side of the tongue and a fully developed paralysis of the right facial muscles were present by the fifth day. The right lower eyelid began to droop considerably; there-

fore, a right blepharoplasty was performed on the 10th day. Rapid, progressive, and severe bilateral deafness began on the 16th day. Because it was believed that the symptoms were due to involvement of the posterior fossa by mycosis fungoides, 700 r of x-radiation was given to the right ear during the succeeding four days. At the completion of this treatment, both sides of the tongue were devoid of sensation anteriorly, and the mouth was dry because of involvement of the chorda tympani. Finally, on the 23d day, complete left facial paralysis and hyperesthesia of the left ear occurred. Without delay, with use of a 10×10 cm. field centered over the external auditory canal, 600 r of x-radiation was given to the left ear. After this therapy the left eyelid improved within four days, as did the facial weakness, and the hearing returned within eight days. The right facial paralysis and deafness on the right began to improve within one month after x-ray treatment was begun. The right facial weakness and difficulty in drinking were still present six months later. In retrospect, it is obvious that the fear of producing edema about the nerve in bony inelastic canals was not adequate reason for the delay in instituting radiation therapy. This was his first x-ray treatment in nine years.

After this episode, the itching was worse at night, and during the succeeding year it increased progressively but again diminished without further therapy other than a low-carbohydrate diet. Small skin plaques appeared and regressed spontaneously.

A small recurrent basal-cell carcinoma beneath the right nares was excised in April, 1944. The remaining skin was smooth, with numerous pigmented areas, and itching was minimal throughout this year. During this period of illness, repeated laboratory tests revealed little deviation from normal except in the blood cell count.

During all these years Dr. Cannon remained actively at work, both in the laboratory and in his numerous national commitments. He also traveled extensively. In March, 1945, toward the end of a three months' stay in Mexico, doing research, he acquired what was thought to be a mycotic infection of the feet, which had become infected with pyogenic organisms. At about the same time his general physical state began to fail, and the scratching of his skin may have spread the infection. By April his hyperkeratotic feet were covered with pustules; these were also present on his face, and most of his skin was very red, thickened, and dry. For the first time, the liver and spleen were palpable on deep inspiration and there was some enlargement of the lymph nodes in both groins. Anemia also made its first appearance, with a blood hemoglobin of 10.9 gm. per 100 cc. and a red cell count of 3,800,000 per cubic millimeter. Because of the apparent progression of the disease, spray irradiation was given over a four-day period with unfiltered 100 kv. x-rays in doses of 20 r per day to include the torso and upper legs. Ten days later the itching was lessened, but it was uncertain whether the x-radiation or the control of the skin infection was responsible for the general improvement.

Within two weeks, recurrent infection of the skin occurred and Dr. Cannon reentered the hospital. There were numerous pustules, mostly on his hands and feet. The predominant organisms were hemolytic staphylococci and beta-hemolytic streptococci. Small hemorrhagic areas also appeared on the feet. Laboratory studies revealed blood hemoglobin of 9.6 gm., plasma protein 6.9 gm., and NPN 42 mg. per 100 cc. Albumin appeared in the urine. Transfusions, antibiotics, and supplementary measures were employed to control the infection. He was somnolent for some days. The liver, spleen, and lymph nodes could not be palpated. Finally, between June 22 and 30, x-radiation in single doses of 100 r per day was given to the back, chest, thighs, and both arms, for a total of 700 r, while the soles of his feet received a total of 220 r (100 kv., 10 ma. unfiltered x-rays with an open portal at 24 cm. distance were used).

The skin healed rapidly. The hemoglobin, however, dropped to 9.0 gm. per 100 cc. The reticulocyte count was 1.2%, and there was a normal percentage of polymorpho-

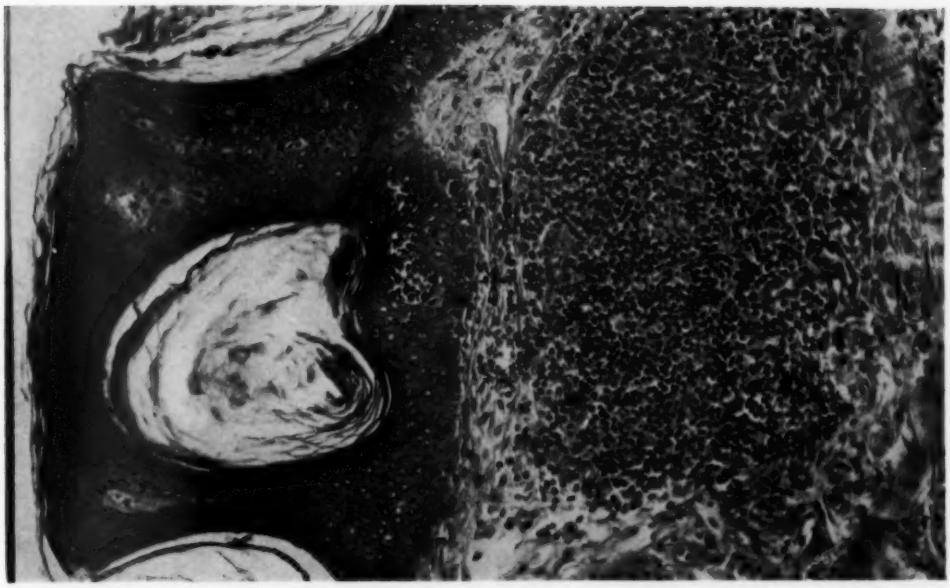


Fig. 2.—1932 biopsy specimen. There is a focus of cellular infiltration in the corium. The epidermis is acanthotic and hyperkeratotic. Modified Giemsa stain; × 155.

nuclear leucocytes. The blood nonprotein nitrogen rose to 59 mg. per 100 cc. Albumin was now constantly found in the urine. He left the hospital on July 19, clinically very much improved.

Three enlarged lymph nodes could be felt in the left groin on Sept. 5, 1945, but the liver and spleen were not palpable. The skin was soft, with normal color and practically without scaling, but the itching was intense. Marked anemia was present, the hemoglobin level being only 6.5 gm. per 100 cc. and the red cell count 2,800,000 per cubic millimeter.

During that month dyspnea occurred, weakness became severe, and the liver became palpable. On Oct. 1, 1945, at the age of 74, Dr. Cannon died of intercurrent pulmonary infection. The mycosis fungoides had been present for more than 14 years.

PATHOLOGIC FINDINGS

Methods.—At the time of each biopsy, a pathologist was present in the operating room with a large bottle of Zenker's fluid containing 5% acetic acid, into which the specimen was dropped by the surgeon. After preliminary fixation for half an hour, very thin blocks were fixed further in acetic acid and Zenker's fluid. These were dehydrated as rapidly as possible, cleared in cedar oil, embedded in paraffin, and stained routinely with a modified Giemsa stain. On selected blocks, sections were prepared with Mallory's phosphotungstic acid and hematoxylin, Mallory's aniline blue connective tissue stain, Foot's method for reticulum, and Weigert's stain for elastic tissue. These sections have been placed on file at the Armed Forces Institute of Pathology as Accession No. 658356.

In view of the relation of the visceral lesions at necropsy to the skin lesions endured by Dr. Cannon for many years, an account of the histology of the skin at various periods before and after death follows:

Biopsy Findings.—1932: Slides were submitted to leading dermatologists at various medical centers, all of whom were in accord with the diagnosis of lymphoblastoma, and almost all of them agreed that the lesion was a premycotic stage of mycosis fungoides. The sections included skin with and without seborrheic keratosis (Fig. 2). All sections were characterized by many foci of cellular infiltration, which were found chiefly in the upper layer of the corium, occasionally surrounding hair follicles deep in the corium, and sometimes in the papillary layer. These foci were sharply circumscribed. They were

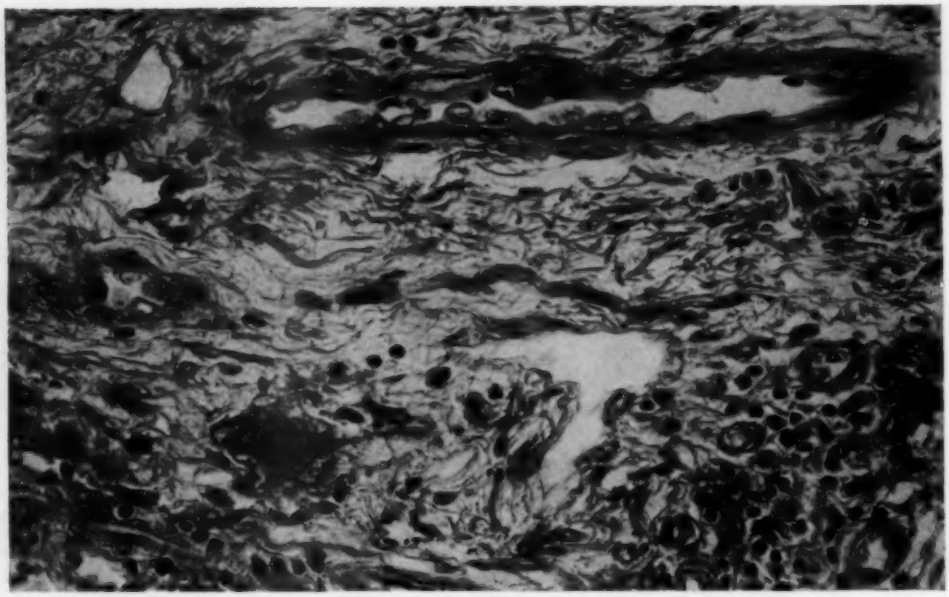
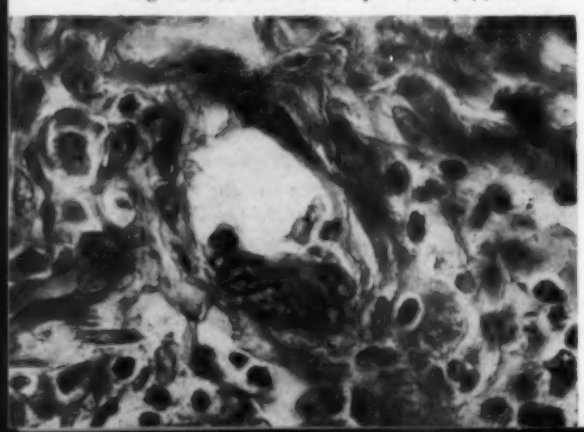


Fig. 3.—1932 biopsy specimen. A giant cell is seen in the lumen of a venule. The adjacent endothelial cells are large and swollen. Another giant cell lies near, but not within, a small vessel. Modified Giemsa stain; × 400.

circular, oval, or irregular in outline and occasionally formed bands of considerable length at the level of the subpapillary plexus of blood vessels. Generally the foci contained one or more blood vessels of precapillary size.

The cells varied in appearance and represented several types. Lymphocytes at various stages of development and monocytes were identified. Mast cells were occasionally found, but polymorphonuclear leucocytes were usually absent. Rarely, a phagocyte containing an ingested chromatin particle or a degenerated nucleus was present. Mitotic figures were numerous. Connective tissue and reticulum stains showed a rather delicate collagenous network. Cells with very finely granular cytoplasm and relatively large nuclei with sharp nuclear membranes and prominent nucleoli were numerous. Multinucleated cells, often of considerable size, were common. In general, they lay adjacent to blood vessels, were often elongated, and invariably had processes which usually extended to collagen fibers. They measured from 20μ to 80μ in diameter. The cytoplasm was very finely granular and faintly acidophilic, and it contained varying numbers of clusters of centrioles. The nuclei of the giant cells were oval and had prominent membranes, large nucleoli, and variable amounts of finely divided chromatin. In each cell one or more nuclei were hyperchromatic. Frequently the cell boundary was deeply indented or separated. In the concavities thus formed there were round cells of the types predominating in these lesions. The appearance of the cells may be interpreted equally well as the incorporation of smaller cells by the giant cells or as a breaking of the giant cells into mononuclear cells.

Fig. 4.—1932 biopsy specimen. A giant cell is present within a venule. The character of the cells in the infiltrate is also shown. Mallory's phosphotungstic acid and hematoxylin stain; × 630.



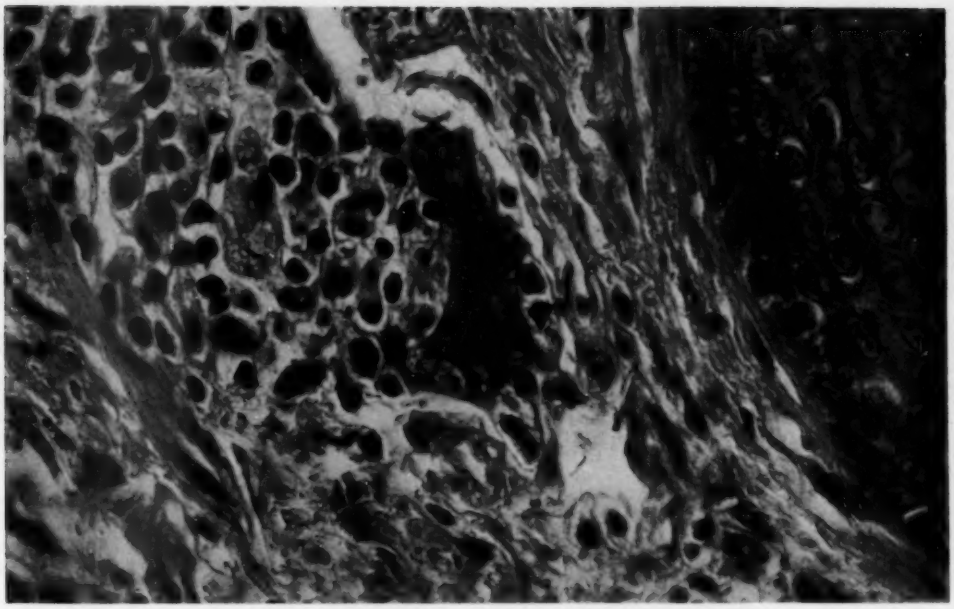


Fig. 5.—1934 biopsy specimen. The appearance of a giant cell in the corium is illustrated, as well as the character of the infiltrating cells. Modified Giemsa stain; × 1000.

Similar cells lay in the lumens of veins, which indicated that they might have arisen from the endothelium (Figs. 3 and 4). The epidermis was moderately thickened (acanthotic) and hyperkeratotic.

1934: There were foci of cellular infiltration and giant cells identical with those of the 1932 biopsy specimens (Fig. 5).

1935: This specimen included normal skin adjacent to a red area. All parts, including the

Fig. 6.—1935 biopsy specimen. The mycosis fungoides lesions are more active and invasive than in 1932 (Fig. 2). Modified Giemsa stain; × 120.

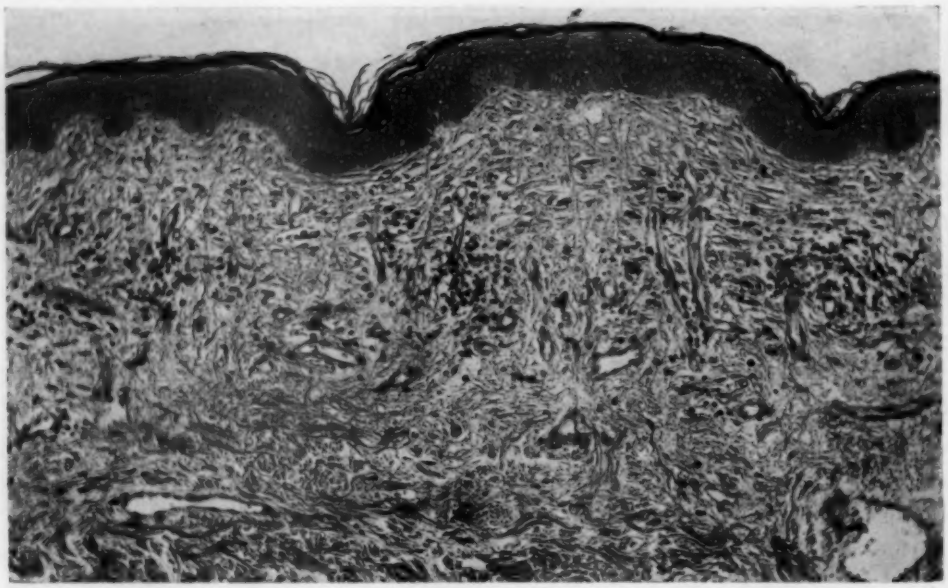
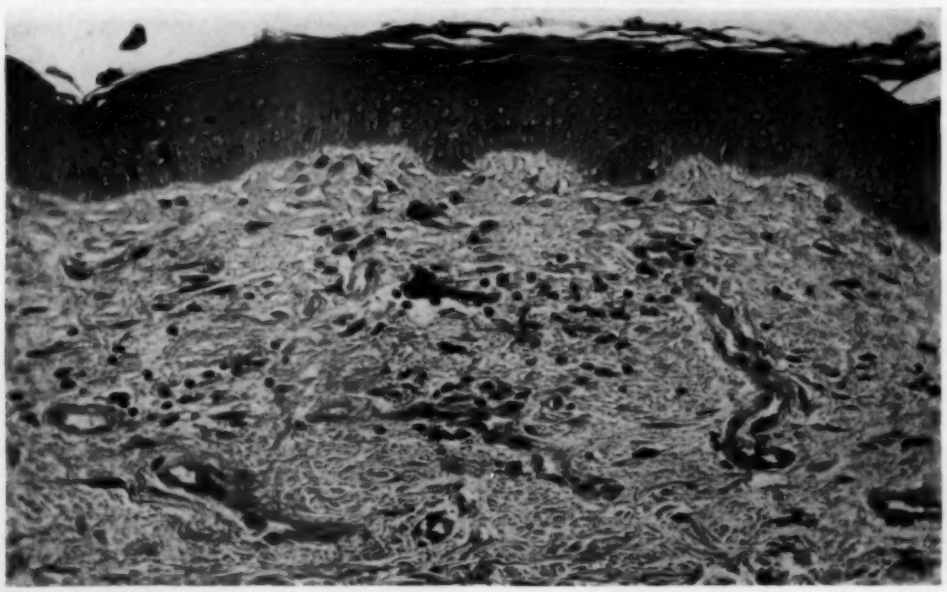


Fig. 7.—1944 (November) biopsy specimen. The process is in a stage of remission and repair. Note the absence of papillae, the loose-textured and richly vascular corium, and the decrease in infiltrating cells. Modified Giemsa stain; × 125.

uppermost layer of this corium and the papillae, were heavily infiltrated with cells identical with those described in the 1932 biopsy specimens. In the end of the section toward the area which appeared red clinically, the papillary capillaries were engorged, red blood corpuscles had extravasated, and a fibrin network lay beneath the epidermis covering

Fig. 8.—1944 (November) biopsy specimen. A single giant cell and a few infiltrating cells are still present, though the process is in remission. Modified Giemsa stain; × 210.



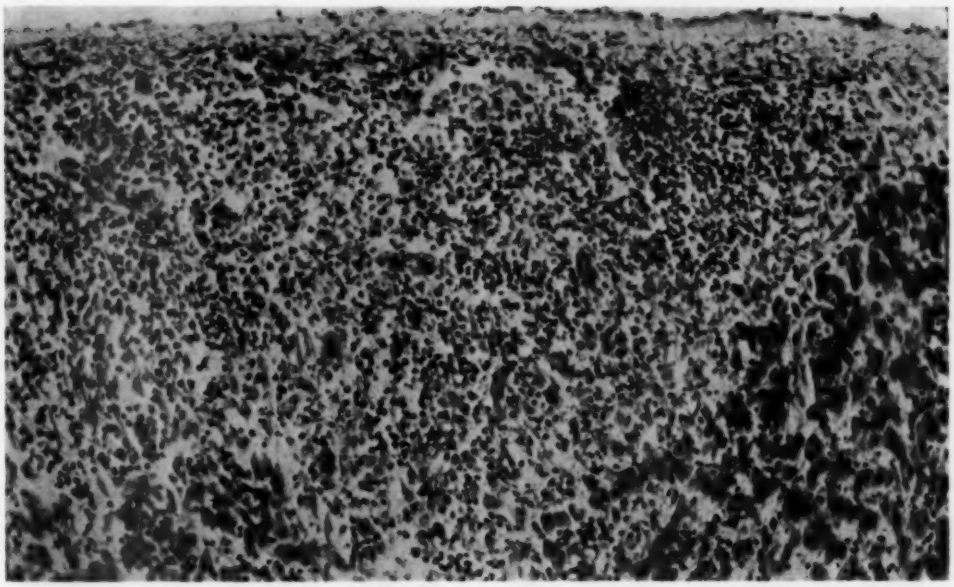
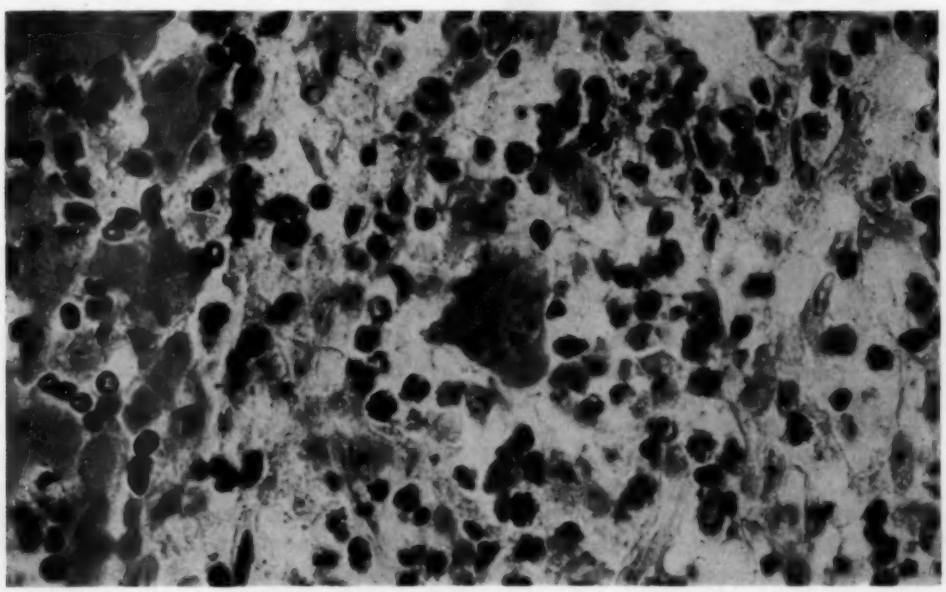
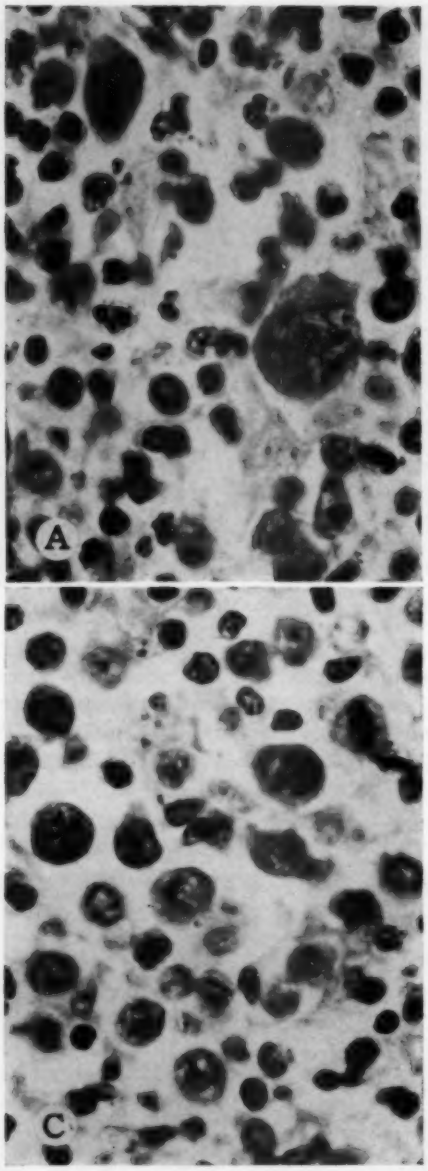


Fig. 9.—Necropsy material, 1945. A nodular lesion in the liver. Modified Giemsa stain; × 150.

the papillae. The overlying epidermis was very thin, and the Malpighian layer had disappeared. What remained was a small layer of sparse prickle cells and imperfectly cornified cells (Fig. 6). Scattered groups of a few round cells occupied sharply demarcated circular cavities in the epidermis. The giant cells described in previous sections

Fig. 10.—Necropsy material, 1945. Higher power view of a nodular lesion in the liver to show the similarity of the cells to those in the skin lesions. Modified Giemsa stain; × 550.





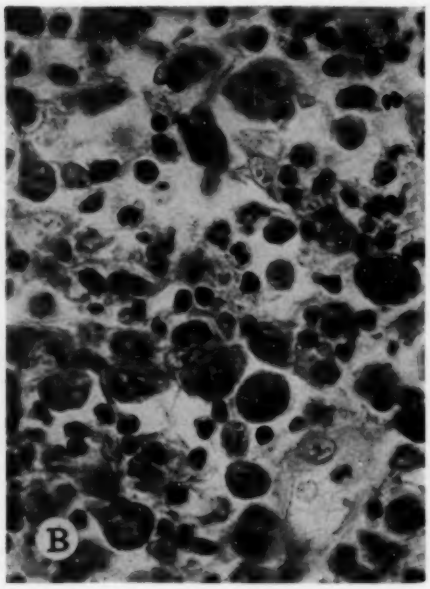


Fig. 11.—Necropsy material, 1945. Three fields from lymph nodes in the right axilla. Modified Giemsa stain; × 860. *A*, giant cells are shown. *B*, cells similar to those in the skin lesions replace the lymphoid follicles. *C*, details of the cells are shown; there are two mitoses in this field.

were few and small. The impression was that of a more active and invasive process than those shown in the 1932 and 1934 biopsy specimens.

1937: This skin was from a region of precancerous chronic x-ray dermatitis on the palm, showing great hyperplasia and hyperkeratosis of the epidermis. The upper corium was dense, but foci comparable to those of the 1932 and 1934 biopsy specimens were present.

1940: A small invasive epidermoid carcinoma.

1941: Numerous patches of x-ray dermatitis were excised from the hands. All sections showed some degree of the process under study in spite of the density of the collagen in the upper corium.

1944 (March): A lesion removed from the upper lip was a typical basal-cell carcinoma.

1944 (November): From its appearance this specimen could be interpreted only as resulting from remission and repair (Figs. 7 and 8). The epidermis was of normal thickness but definitely hyperkeratotic. The papillae of the corium were absent in many regions. The corium beneath the epidermis was loose-textured and vascular, as if the papillary capillaries had remained and become dilated. At the level of the subpapillary plexus and above were scattered round cells of various types, and occasionally grouped

about a dilated vessel were small collections of cells such as those described in the original specimen. Many cells had densely stained, presumably pyknotic, nuclei, but there were cells of larger size with hyperchromatic nuclei. An occasional giant cell was found adjacent to these foci but entirely surrounded by connective tissue, often with fairly dense collagen (Fig. 8). Occasional cells (as in all the skin biopsy specimens) contained brown pigment granules, presumably melanin.

Necropsy.—The skin of the entire body appeared thickened, dry, and scaly with numerous excoriations. Microscopically, all sections showed evidence of remission of the process. All had dense, hyaline collagen in the upper corium, with regions of obliteration of the papillae. There were diffuse infiltration with round cells and occasional foci the size of miliary tubercles about vessels of the subpapillary plexus. The cells were like those of the more active skin lesions of 1932 and 1934. Giant cells of the type already described were present, usually lying in the dense collagen adjacent to the foci of infiltration. These cells had less cytoplasm than those within the foci of round cells; also, the nuclei were smaller and less densely stained. Mitoses were not found. Of interest in these sections of sclerosed skin was the absence of cells characteristic of inflammatory processes.

Numerous firm tumor-like nodules ranging from miliary tubercle size to 2.0 cm. in diameter were scattered extensively throughout the lungs and in the abdominal viscera (Fig. 9), lymph nodes, and bone marrow. The spleen was three times the normal size. In general, the characteristics of the infiltrating cells in the nodules were identical with those described in the skin (Fig. 10). There were vast areas of consolidation in the lower lobes of the lung due to a severe pneumonic process suggestive of a viral type succeeded by bacterial invasion. Small foci of the process described in the report of the skin biopsies were also present, and these were undergoing necrosis. All the involved lymph nodes revealed an absence of lymphoid follicles, very little obliteration of lymph sinuses, and no infiltration of the capsules of the nodes (Figs. 11 and 12). The increase in size of the lymph nodes was the result of the accumulation of large cells in the lymphoid cords or medulla. In the bone marrow there were lesions similar to those seen elsewhere, characterized by autolysis of abnormal cell aggregates, while the marrow as a whole was actively erythroblastic and myeloblastic.

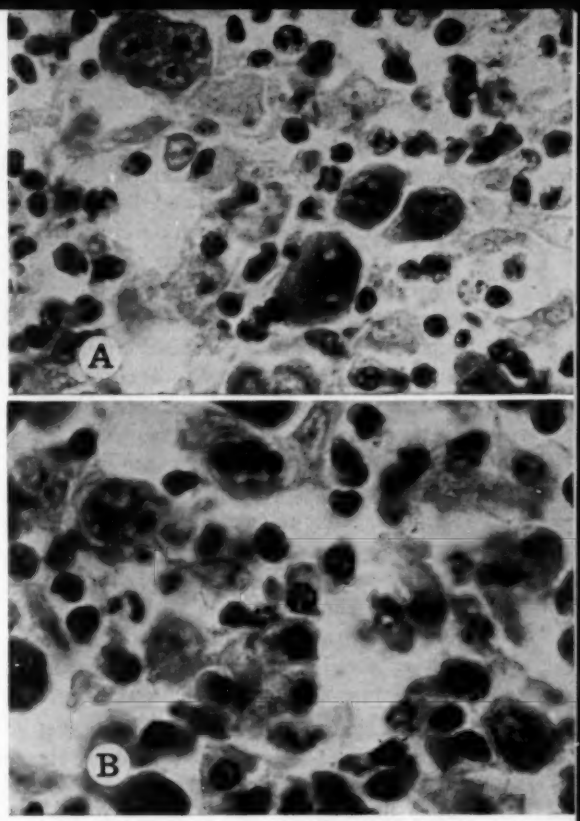


Fig. 12.—Necropsy material, 1945. Two fields from lymph nodes in the left axilla. *A*, infiltration with cells similar to those in the skin lesions, including giant cells. No lymphoid follicles are present. Modified Giemsa stain; × 740. *B*, preservation of lymph sinuses in spite of the abnormal cellular infiltration. Modified Giemsa stain; × 1000.

Final Diagnosis.—Mycosis fungoides involving the skin, lungs, myocardium, liver, spleen, intestine, bone marrow, and lymph nodes. An active febrile illness with bronchopneumonia terminated life, whereas the disease of long standing was in a period of remission at the time of death.

COMMENT

Four neoplastic diseases arose during the last 14 years of Dr. Cannon's life: basal-cell carcinomas, an epidermoid carcinoma, my-

cosis fungoides, and leukemia. Etiologic agents can only be postulated, but a predisposing factor may have been the considerable exposure to unfiltered x-ray radiation suffered in early investigative work on the gastrointestinal tract. The very prolonged exposure to radiation resulted in an x-ray dermatitis of the hands, keratosis, and, finally, multiple carcinomas. The histology of the dermatitis and the sequences antecedent to malignant change are similar to those described elsewhere in other early workers with x-ray apparatus.[7] The mycosis fungoides with a leukemic phase was possibly, but less certainly, related to this irradiation. From the clinical point of view, the mycosis fungoides proved to be the greater problem. During the 14-year course of this disease, many interesting phenomena were manifest:

First, the spontaneous remissions that so often occurred, especially in the early stages of the disease. The skin plaques and itching often disappeared, only to reappear later.

Second, the slow rise in the white blood cell count to 32,700 per cubic millimeter with a concomitant absolute increase in the lymphocytes to a peak of 90%. A gradual remission followed, but it was not until four years later that the differential count approached normal. This increase in the white blood cell count represented a leukemic phase of mycosis fungoides, emphasizing the close relationship of this disease to the lymphomas and leukemias.

Third, the development of bilateral facial paralysis and deafness. Apparently this was the result of cellular infiltration in the posterior fossa near the internal auditory meatus, which involved the auditory nerve, chorda tympani, and facial nerves. Fortunately this condition responded to irradiation.

Dr. Cannon's scientific interest led to a continuous exploration of new agents which might improve his condition. Ketogenic and low- and high-carbohydrate, fat, or protein diets were employed, without improvement. Neither did a vaccine of Escherichia coli cultured from the urine aid the disease. Vitamins A and D were found to be of value and were constantly administered. With other patients, however, these measures have been relatively unsuccessful. On three separate occasions x-ray therapy proved to be of palliative value, chiefly in relief of itching. The proper management of x-ray therapy in a patient already suffering from the effects of x-irradiation presented a considerable challenge.

Part of the value of the pathologic material lay in the selection of time and site of each biopsy specimen by the pathologist so that known appearances of the clinical lesions could be related to the histologic evidence of progression or remission. In addition, it provided an opportunity to explore the histogenesis of certain cellular elements of mycosis fungoides.

That the name mycosis fungoides applies to the cutaneous manifestations of Dr. Cannon's disease cannot be disputed; yet that name throws no clear light on the nature of the process or upon the origin of the cells composing the lesions. The appearance of the cells with large hyperchromatic nuclei, numerous mitoses, absence of polymorphonuclear leucocytes, and great scarcity of mature lymphoid and plasma cells indicates a neoplastic rather than an inflammatory or reactive process. The lesions of the corium of the skin, which at first suggested inflammatory response, had an entirely different cellular composition from either acute or chronic inflammatory lesions of the skin. The absence of follicles and disruption of architecture in the lymph nodes again indicated a neoplastic process.

The presence of a distinctive type of giant cell seemed to be an essential component of the process. These giant cells were unlike foreign-body giant cells or the multinucleated cells of Hodgkin's disease; in fact, they seemed to be almost pathognomonic of mycosis fungoides. Very rarely such giant cells have been seen in inflammatory skin lesions and in senile skin with "elastosis."

The histogenesis of these giant cells has been ascribed to fusion of reticular or connective-tissue cells, an explanation not com-

patible with the observations in the present material. Postulation of the origin of the giant cells in immature reticulum cells is also at variance with our findings. In carefully prepared sections obtained over a period of 14 years, there was no evidence that the multinucleated cells produced fibrils. On the contrary, they were always free from reticulum and collagen fibers.

The presence of fully formed giant cells in sites such as the lumens of veins and in direct continuity with the endothelium (Figs. 3 and 4) suggests that they are of endothelial origin. The formation of other types of giant cells from these cells is well known. Endothelial cells have the capacity of ameboid movement. It is possible, therefore, that the giant cells of endothelial origin can lose their vascular connections by migration. Giant cells could also result from the fusion of endothelial cells which had migrated as separate units. Such special characteristics as are acquired by the different types of giant cells arising in endothelium are perhaps due to varying environments created by the etiologic agents of the lesions.

While on very rare occasions giant cells similar to those in mycosis fungoides have been seen in senile skin with elastosis, there is no evidence from the present material that elastic tissue elements are concerned with the production of the giant cells in mycosis fungoides. No fragments of elastic tissue could be found within the giant cells, and the entire lesion was devoid of elastic fibers.

On the basis of this material, it seems a legitimate speculation that mycosis fungoides should not be considered a neoplasm but a reaction (to agents unknown) which, like other known premalignant processes, leads to the development of neoplastic properties in responsive cells.

SUMMARY

This report discusses a patient with mycosis fungoides who was followed for 14 years.

Bilateral facial paralysis and a temporary lymphatic leukemia are complications which developed in the course of the disease.

Successive biopsies and the necropsy allow study of the progression of the lesions through exacerbations and remissions.

Evidence is presented that the giant cells in mycosis fungoides are not related to reticulum or collagen fibers and that they are probably derived directly from the endothelium of blood vessels.

REFERENCES

1. Gall, E. A., and Mallory, T. B.: Malignant Lymphoma, Am. J. Path. **18**:381, 1942.

2. Warthin, A. S.: Genetic Neoplastic Relationships of Hodgkin's Disease, Aleukaemic and Leukaemic Lymphoblastoma and Mycosis Fungoides, Ann. Surg. **93**:153, 1931.

3. Lever, W. F.: Histopathology of the Skin, Ed. 2, Philadelphia, J. B. Lippincott Company, 1954.

4. Cawley, E. P.; Curtis, A. C., and Leach, J. E. K.: Is Mycosis Fungoides a Reticuloendothelial Neoplastic Entity? A. M. A. Arch. Dermat. & Syph. **64**:255, 1951.

5. Heite, H. J., and Socha, P.: Häufigkeitsanalytische Untersuchungen zur Symptomatologie der Mycosis fungoides, Arch. Dermat. u. Syph. **193**:118, 1951.

6. Wolbach, S. B.: Studies on Rocky Mountain Spotted Fever, J. M. Res. **41**:1, 1919, p. 76.

7. Wolbach, S. B.: Pathological Histology of Chronic X-Ray Dermatitis and Early X-Ray Carcinoma, J. M. Res. **21**:415, 1909.

8. Garb, J., and Sims, C. F.: Reticuloendothelial Cell Hyperplasia in Relation to Antimonial Therapy of Mycosis Fungoides, A. M. A. Arch. Dermat. & Syph. **68**:1, 1953.

Experimental Proteinuria

An Electrophoretic Study of Three Different Types in Dogs

ROBERT D. COYE Jr., M.D., Madison, Wis.
DAVID L. MAUDE, B.A.
ROBERT F. DIBBLE, Rochester, N. Y.
and
CHARLES L. YUILE, M.D., Los Angeles

Within recent years the presence of protein in the urine has been recognized by most workers to be the result of either increased glomerular filtration or decreased tubular reabsorption of protein, or a combination of the two factors.[1] This would imply that normally all but a minute quantity of any protein which is filtered by the glomerulus is reabsorbed by the tubular epithelium. Electrophoretic separation has shown that the small amounts of protein normally present in the urine of rats [2] and man [3] contain α- and β-globulins but relatively little albumin. The globulins in general have much greater molecular weights than albumin, and molecular size is one of the major factors determining rate of transfer of colloids across semipermeable membranes. While methods for direct measurement of proteins in glomerular filtrate are not available, it is assumed that relatively greater amounts of albumin are normally filtered and therefore quantitatively reabsorbed by the kidney.

Received for publication Aug. 5, 1955.

From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

This work has been supported in part by funds from the Office of the Surgeon General. Aid was also received from the Ernest L. Woodward Fund.

Present address: Department of Pathology, University of Wisconsin Medical School (Dr. Coye); Department of Pathology, University of California School of Medicine at Los Angeles (Dr. Yuile).

The newer methods of electrophoresis in starch [4] and on filter paper [5] provide opportunities for studies on blood and urine protein fractions not previously considered practical. The experimental work reported here was undertaken in an attempt to characterize three types of experimental proteinuria with respect to the nature of the protein excreted, possible changes in the relative amounts of the various components with time, and the relationship between serum and urinary proteins. Proteinuria was induced in one dog by repeated injections of plasma as originally described by Terry, Hawkins, Church, and Whipple.[6] In six dogs proteinuria was produced by intravenous injections of $HgCl_2$. Two of these dogs received a second injection of $HgCl_2$ after lapses of five and eight weeks. A third type of proteinuria was produced in four additional dogs by intravenous administration of renin.

It is of interest that there is considerable variation in the proportion of albumin and globulin in the urine both in the experimental proteinurias described and also in various types of human proteinuria of different etiology.*

METHODS

General.—The experimental animals were normal dogs not previously used for experimental purposes. They were fed a standard kennel ration before and during the experiments. Water intake during the experimental period was limited to slightly more than the amount of urine excreted. Control urine sediment examinations and tests for urinary protein as described below were within normal limits for all dogs.

Urine.—Voided urine was collected in metabolism cages in some cases; in others, urine was obtained by catheterization. When sufficient urine was availa-

* References 7 through 11.

ble, a heat and acetic acid test gave a very crude estimate of the protein concentration. In general, it was found that if no distinct precipitate was formed with trichloroacetic acid or heat and acetic acid, the amount of protein present was too small to be measured by the micro-Kjeldahl procedure. The cationic detergent method of Jacox [12] and the modification of the Lowry colorimetric method [13] could not be used for measurement of urinary protein because of interfering substances in the urine.

Blood.—In some instances total serum protein determinations were done by the macro-Kjeldahl method, and albumin was determined by the method of Majoor [14] after precipitation of the globulin with 26.8% Na_2SO_4. In other cases the proteins were separated into three fractions (total protein, total globulin, and α-globulins) by the Jacox detergent method.† Blood urea nitrogen determinations were done by titration of ammonia released by urease.

Electrophoresis.—Filtered and dialyzed urine was lyophilized, and the protein was subjected to zone electrophoresis with the use of either starch blocks or filter paper. The protein was eluted from 1 cm. segments of the starch blocks with the use of saline and measured by the Lowry method.[13] The filter paper strips were dyed with bromophenol blue, and the dye intensity was measured either directly with a Photovolt Densitometer or by photoelectric colorimetry after dye elution. It should be noted that since the electrophoretic patterns were prepared on varying amounts of urine, comparisons of different curves are valid only for qualitative differences in protein composition and not for relative total protein content.

I^{131}-labeled dog serum protein was prepared by Drs. W. Bale and I. Spar according to methods described by them.[15] Tracer doses of this material were given intravenously to several dogs at the height of proteinuria. Specific activities were calculated for plasma and urinary protein fractions electrophoretically separated in starch. A sodium iodide scintillation counter was used for radioactivity measurement.

Renin.—Renin used in these experiments was provided by Dr. Harry Goldblatt. The renin had an activity of 6.7 dog units per milligram of dried powder. All injections were intravenous.

EXPERIMENTAL OBSERVATIONS

I. *Hyperproteinemia.*—Dog 51-22, a mongrel female beagle weighing 13.5 kg., with an initial total protein of 6.4 gm. per 100 ml., was given daily injections of 200 to 300 ml. of pooled dog plasma for 22 days, and 400 to 500 ml. of pooled plasma every 2 to 3 days during the following 18 days. Protein was first found in the urine on the eighth day, when the plasma protein concentration had reached 9.16 gm. per 100 ml. Protein continued to appear in the urine for the duration of the experiment, with a peak level of 0.8 gm. per 100 ml. and a 24-hour excretion of 13.9 gm. On the 40th experimental day the dog was given a tracer dose of I^{131}-labeled dog serum intravenously. The total protein was 9.5 gm. per 100 ml. with an A/G ratio of 5.3/4.2. Proteins from a two-hour urine sample and an intermediate serum sample were electrophoretically separated in starch. Specific activities for each segment were calculated as the per cent of the total dose per milligram of protein. The specific activities of the urine and plasma proteins were almost identical. The electrophoretic pattern of the urinary protein showed small quantities of serum globulins and a very high peak in the albumin range.

This dog was killed at the end of the experiment. The kidneys on gross examination were slightly swollen and pale, and microscopic sections revealed considerable granularity and fraying of the lightly stained proximal tubular epithelium, with scattered hyaline casts in the distal tubules.

II. *Mercuric Chloride Poisoning.*—The results of these experiments are summarized in Tables 1, 2, and 3. As shown in Table 1, the doses of $HgCl_2$ ranged from 1.5 to 4.1 mg. per kilogram of body weight. The two dogs receiving 1.5 mg/kg. and the one dog receiving 2.0 mg/kg. survived with only moderate evidence of intoxication. The remainder developed uremia and oliguria and were killed when moribund 3 to 10 days after the $HgCl_2$ injection.

Typical changes noted in the serum and urinary proteins for each experiment are shown in Tables 2 and 3. In all but one of the dogs in which serum α-globulins were determined, there was a marked early elevation in this fraction following $HgCl_2$ injection. The electrophoretic patterns shown in Figure 1 illustrate this serum α-globulin rise in a typical experiment. Some elevation of this fraction is still noted three weeks after

† Dr. Ralph Jacox made these determinations.

TABLE 1.—*General Results of Intravenous $HgCl_2$ Injection in Dogs*

Dog	Sex	Wt., in Kg.	Dose, Mg/Kg.	Experimental Result	Terminal BUN, Mg/100 Ml.
52-17	F	9.1	2.0	Uremia, oliguria; killed on 10th day	374
51-194	M	9.8	1.5	Moderate diuresis, lethargic, bloody stool; survived	9
51-194	M	9.6	3.0	Uremia,* oliguria; killed on 6th day	123
53-389	M	17.0	3.0	Uremia, oliguria, bloody stool; killed on 3d day	159
54-147	F	9.1	4.1	Uremia, oliguria, pregnant; killed on 3d day	64
54-341	M	15.5	1.5	Lethargic, diuresis; survived	...
54-341	M	14.0	2.0	Marked diuresis †; survived	...
54-264	M	16.6	2.0	Uremia, anuria; killed on 6th day	...

* 2½ months after first dose.
† 5 weeks after first dose.

TABLE 2.—*Serum Proteins Before and After $HgCl_2$ Injection*

		Initial			Postinjection			
Dog	$HgCl_2$, Mg/Kg.	Total Protein, Gm/100 Ml.	A/G Ratio	α_1- and α_2-Globulins, Gm/100 Ml.	Total Protein, Gm/100 Ml.	A/G Ratio	α_1- and α_2-Globulins, Gm/100 Ml.	Time
52-17	2.0	5.65	.....	...	...	.....	....	....
51-194	1.5	7.5	4.4/3.0	...	6.9	3.2/3.7	....	72 hr.
51-194	3.0	6.4	3.8/2.6	1.7	6.7	4.0/2.7	2.65	6 days
53-389	3.0	5.5	3.2/2.3	2.0	6.5	3.0/3.5	2.8	72 hr.
54-147	4.1	5.3	3.3/2.0	1.9	6.7	3.0/3.7	3.0	24 hr.
54-341	1.5	5.9	2.5/3.4	1.7	5.9	3.2/2.7	2.6	6 days
54-341	2.0	6.0	2.8/3.2	2.7	7.5	3.8/3.7	3.0	24 hr.
54-264	2.0	6.6	4.0/2.6	1.8	7.0	4.6/2.4	1.8	12 hr.

TABLE 3.—*Urinary Protein Composition Following $HgCl_2$ Injection*

	Early Specimen			Late Specimen		
Dog	Albumin, Per Cent	Globulin, Per Cent	Time	Albumin, Per Cent	Globulin, Per Cent	Time
52-17	0.22 gm.%*		1-24 hr.	0.015 gm.%*		48-72 hr.
51-194	10	90†	3-10 hr.	50	50	27-43 hr.
51-194	17	83†	4-9 hr.	33	67	9-19 hr.
53-389	25	75†	1-5 hr.	43	57	10-24 hr.
54-147	45	55†	1-5 hr.	0	0	.....
54-341	11	89‡	7 hr.	44	56	7 days
54-341	31	69‡	1 hr.	52	48	30 hr.
54-264	24	76‡	3-6 hr.	12	88	8-18 hr.

* Not separated.
† Predominantly α-globulin.
‡ Predominantly β-globulin.

$HgCl_2$ injection. Also noted in Table 3 is the fact that urinary protein, early in the course of the proteinuria, consists predominantly of globulin. In several instances the protein was almost exclusively α-globulin. In all but one dog (54-264) the percentage of albumin in the urinary protein increased significantly with time. Figure 2 graphically illustrates this sequence of events in Dog 54-341.

Dogs 54-147 and 54-264 were given tracer doses of homologous I^{131}-labeled serum protein intravenously during periods of marked proteinuria following $HgCl_2$ injection. Specific activities of serum and urinary protein collected at approximately the same time were compared after electrophoretic separation. The results obtained, while not entirely satisfactory, strongly suggest that the urinary protein is largely, if not completely, derived from the serum proteins.

Autopsy Findings: Moribund dogs were killed with a few milliliters of intravenous chloroform. Sections of tissue were fixed in Zenker's fluid or 10% formalin and stained with hematoxylin and eosin. All dogs

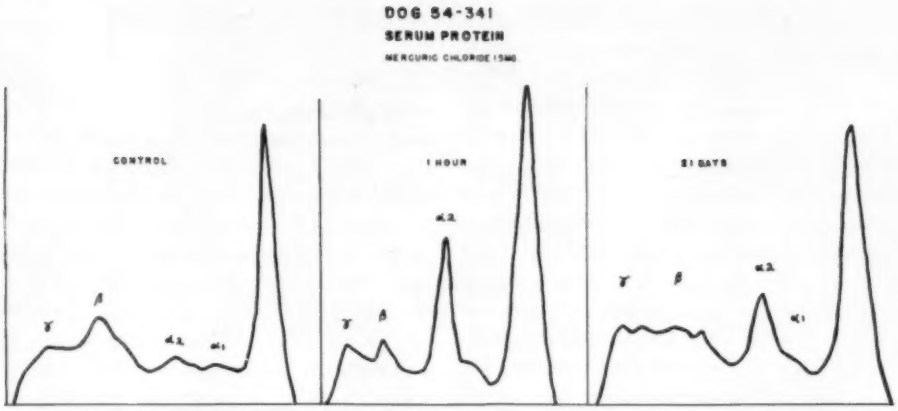


Fig. 1.—Paper electrophoretic separation of dog serum proteins before and after intravenous injection of mercuric chloride. After administration of this chemical there is a prompt rise in α-globulin, and some elevation of this serum protein fraction is still noted at three weeks.

exhibited uniformly enlarged pale kidneys. Microscopic sections showed the usual renal effects of $HgCl_2$ with varying degrees of necrosis of tubular epithelium, chiefly in the proximal portions. The dogs killed before the sixth day showed no regeneration. The glomeruli were uniformly blood-filled. Several dogs showed colitis, and some manifested equivocal atrophy of lymphatic tissue.

III. *Renin Injections.*—Urinary and serum proteins from four dogs, two of which had repeat doses, were subjected to filter paper electrophoresis after the animals had received renin by intravenous injection. Serum proteins were within normal limits in these dogs, and there were no changes in the various protein fractions during the experiments. At least three urine samples collected after renin administration were subjected to electrophoresis in each experiment. Figure 3 illustrates typical results obtained. The control urine of Dog 55-13 showed both globulins and albumin without any striking change in composition after administration of 100 units of renin. The administration of 40 units of renin one week earlier in this dog gave essentially similar results. The control urine of Dog 55-6 showed almost no albumin, and after renin administration the urinary protein consisted of large amounts of γ- and β-globulin with relatively little albumin. The electrophoretic pattern of the control urine of Dog 55-167 was quite similar to that of serum. The postrenin specimens were almost identical in relative protein composition. Dog 55-92

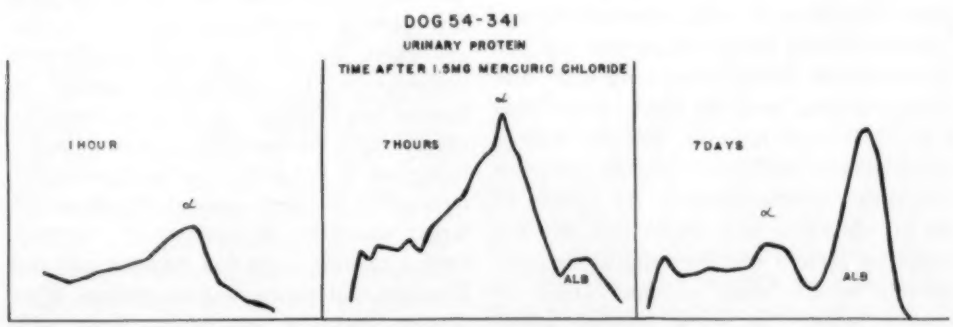


Fig. 2.—Paper electrophoretic patterns of urinary proteins excreted at one hour, seven hours, and seven days after injection of mercuric chloride. Globulins predominate in the early periods, and there is a relatively greater amount of albumin apparent in the curve obtained at seven days.

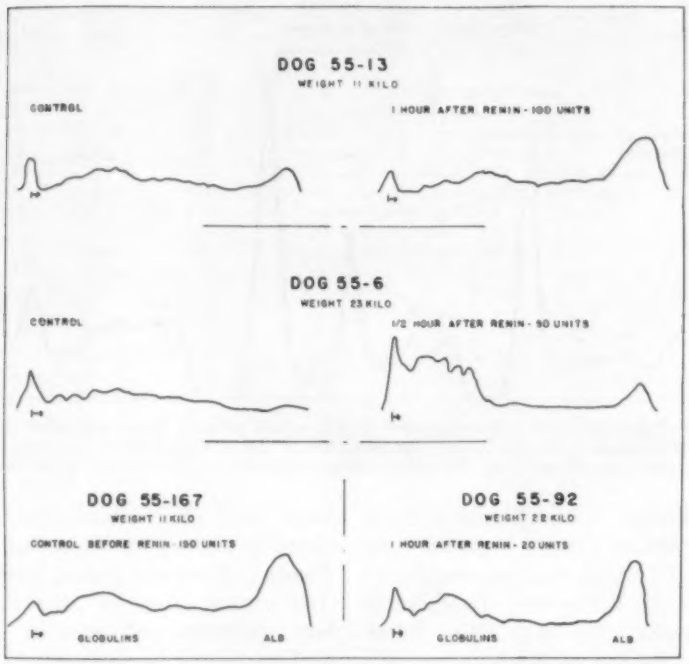


Fig. 3.—Paper electrophoretic patterns of urinary proteins from normal dogs before and after intravenous injection of renin, which increased the total output of urinary protein but caused little qualitative change in its composition. The peaks shown at the origins are partially due to adsorbed protein of unknown nature.

showed principally γ-globulin in the control urine. After administration of 20 units of renin, γ- and β-globulin and albumin were the principal components. A repeat dose of 100 units of renin in this dog after one week gave very similar results. The absolute amount of protein excreted varied considerably during different time periods of each experiment. For example, Dog 55-92, when given 100 units of renin, excreted 60 mg. per hour during the first hour, 115 mg. per hour from the second to the third hour, and 4 mg. per hour from the fourth to the fifth hour. It is clear, however, that the relative composition of the urinary protein remained remarkably constant during the course of the proteinuria in each experiment, and the postrenin protein was very similar in composition to the small amounts found in control urine. The slight changes which occurred with time were associated with an increase in albumin.

COMMENT

Of the three types of proteinuria produced, that due to repeated injections of plasma is probably the best understood.[11] At a plasma level of about 9 gm. per 100 ml., the renal threshold for protein is exceeded and proteins appear in the urine. It is assumed that larger quantities of protein are filtered, and that during the first few days of protein injections the tubular epithelium is capable of nearly quantitative reabsorption. This mechanism appears to become less efficient with time, so that after several days the unabsorbed protein begins to appear in the urine. As the reabsorptive mechanism becomes increasingly saturated, larger quantities of protein are excreted, until a balance is reached between maximal filtration and maximal reabsorption. After several weeks of proteinuria, the protein consists of from 60% to 90% albumin. This high percentage of albumin is believed to

be related to greater filtration of the smaller molecule.

Proteinuria due to $HgCl_2$ poisoning appears to result from two and possibly three types of injury. Tubular necrosis [16] and alterations in renal blood flow [17] are well documented in dogs, and their counterparts have been exhaustively described by Oliver [18] in dogs and in other mammals, including man. MacNider [19] has reported a third type of injury in dogs. He believes that there is some glomerular damage as evidenced by congestion which is sometimes accompanied by exudate, hyaline changes in the endothelium, or thrombus formation. No microscopic evidence of glomerular injury was found in these experiments.

Two distinct phases of proteinuria occur after poisoning with $HgCl_2$. During the first phase urinary protein consists principally of globulins, frequently with a very high percentage of α-globulin. There is a marked elevation in the serum α-globulin fraction corresponding in time with the α-globulinuria. This serum elevation has been described in man and other animals after bacterial infections, fever,[20] malignant neoplasms,[21] and other types of stress. It seems probable that at least some part of the initial globulinuria is due to the sudden elevation of this serum fraction above its renal threshold. In several experiments globulins with the electrophoretic mobility and solubility characteristics of mucoprotein appeared in the urine. It is probable that some part of the α-globulin elevation is due to the presence of proteins of this type.

The theory that larger molecules of protein are reabsorbed in the more distal portions of the proximal tubule is supported by work done with toads with open nephrons.[22] Oliver's work [18] shows some tendency for the tubular necrosis resulting from $HgCl_2$ to localize in this part of the nephron. It is quite possible that the initial injury may selectively affect those portions of the nephron concerned with globulin reabsorption most severely, thus accounting for the early appearance of this protein in the urine.

The second phase of the proteinuria, that of increasing albuminuria, would seem to correspond with the period during which the tubular reabsorptive mechanism is being saturated with albumin. The relative importance of increases in permeability and filtration or destruction of some nephrons with overloading of the remainder as factors in this saturation is not elucidated. Dehydration of these dogs also may play a role in increasing the concentration of protein in the serum, which would also tend to increase the amount of filtered protein that the still-functioning nephrons would attempt to reabsorb. The maximum percentage of albumin in the urine following $HgCl_2$ poisoning (50%) is significantly lower than that with protein loading.

The proteinuria induced by renin in dogs in these experiments is characterized by an increase in the proteins, chiefly globulins, normally present in the urine. In contrast to the other two types of proteinuria described in this paper, about 60% of the urinary protein consists of globulin with some slight tendency to increase the proportion of albumin with time, and there are no associated changes in the serum proteins. While the mechanism of renin proteinuria is in some doubt, the best evidence suggests that it is due to increased capillary permeability.‡ A direct inhibitory effect on tubular reabsorption or a combination of the two factors has also been postulated.[26]

During the course of these experiments several instances of pure globulinuria were found in controls and in dogs shortly after mercury poisoning. One of the renin-injected dogs showed large quantities of globulin and only traces of albumin. No instances of pure albuminuria were found. This point has been emphasized in human proteinuria.[10] It is suggested that inasmuch as globulin filtration is probably less than that of albumin, the reabsorptive mechanism for globulins must be very much less efficient in order for them to appear alone in the urine. Recent work by Spector [27] has

‡ References 23 through 25.

shown a reserve tubular reabsorptive capacity for both albumin and globulins as measured by protein reabsorption in rats given I^{131}-labeled proteins before and after experimental nephritis. According to his work the rat excretes globulins in the urine, even though the tubular reabsorptive mechanism is not "saturated." This would support our belief that the reabsorption of globulin is not as efficient as the reabsorption of albumin. If this is true, the appearance of relatively pure globulin in the urine may be interpreted as being due to changes affecting serum globulin concentration, glomerular permeability, or tubular reabsorptive capacity. These may occur alone or in combination and possibly are of a mild transient nature. The appearance of significant quantities of albumin in the urine, on the other hand, would appear to be a more ominous sign.

SUMMARY

Proteinuria was induced in dogs by three different methods: multiple plasma transfusions leading to hyperproteinemia, mercuric chloride poisoning, and intravenous injection of renin.

Proteins of the serum and urine were fractionated by zone electrophoresis on starch or paper.

The proteinuria associated with hyperproteinemia is characterized by a preponderance of albumin. Inability of the renal tubular epithelium to reabsorb increased amounts of protein filtered through normal glomeruli probably accounts for the appearance of proteins in the urine in this type of experiment.

With mercuric chloride poisoning the urinary protein initially is composed chiefly of globulins, and there is an associated elevation in the serum α-globulin fraction. Urinary protein excreted after the first few hours contains an increasing percentage of albumin. Possible mechanisms involved in the development of this type of proteinuria are discussed.

Renin proteinuria is characterized by an increase in the normally present urinary proteins, which are chiefly globulins. This suggests that renin enhances urinary protein excretion largely by increasing glomerular permeability.

A relationship between qualitative differences in the composition of urinary protein and the severity of the underlying renal disturbance is suggested.

REFERENCES

1. Rather, L. J.: Filtration, Resorption, and Excretion of Protein by the Kidney, Medicine **31**: 357-380, 1952.

2. Sellers, A. L.; Roberts, S.; Rask, I.; Smith, S., III; Marmorston, J., and Goodman, H. C.: Electrophoretic Study of the Urinary Protein in the Rat, J. Exper. Med. **95**:465-472, 1952.

3. Rigas, D. A., and Heller, C. G.: Amount and Nature of Urinary Proteins in Normal Human Subjects, J. Clin. Invest. **30**:853-861, 1951.

4. Kunkel, H. G., and Slater, R. J.: Zone Electrophoresis in a Starch Supporting Medium, Proc. Soc. Exper. Biol. & Med. **80**:42-44, 1952.

5. Slater, R. J., and Kunkel, H. G.: Filter Paper Electrophoresis with Special Reference to Urinary Proteins, J. Lab. & Clin. Med. **41**:619-631, 1953.

6. Terry, R.; Hawkins, D. R.; Church, E. H., and Whipple, G. H.: Proteinuria Related to Hyperproteinemia in Dogs Following Plasma Given Parenterally, J. Exper. Med. **87**:561-573, 1948.

7. Blackman, S. S., Jr., and Davis, B. D.: Electrophoretic and Chemical Analysis of Protein in Nephritic Urine, J. Clin. Invest. **22**:545-549, 1943.

8. Javitt, N. B., and Miller, A. T., Jr.: Mechanism of Exercise Proteinuria, J. Appl. Physiol. **4**:834-839, 1952.

9. Luetscher, J. A., Jr.: Electrophoretic Analysis of Plasma and Urinary Proteins, J. Clin. Invest. **19**: 313-320, 1940.

10. Soulier, J. P.: Étude électrophoretique de 86 cas de protéinurie (albuminurie), Presse méd. **61**: 49-52, 1953.

11. Rifkin, H., and Petermann, M. L.: Serum and Urinary Proteins in Diabetic Glomerulosclerosis: Results of Electrophoretic Analysis, Diabetes **1**:28-32, 1952.

12. Jacox, R. F.: Quantitative Fractionation of Component Proteins of Human Serum with Cationic Detergents, J. Clin. Invest. **32**:661-673, 1953.

13. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L., and Randall, R. J.: Protein Measurement with the Folin Phenol Reagent, J. Biol. Chem. **193**: 265-275, 1951.

14. Majoor, C. L. H.: Possibility of Detecting Individual Proteins in Blood Serum by Differentiation of Solubility Curves in Concentrated Sodium Sulfate Solutions: Comparison of Solubility Curves with Results of Electrophoresis Experiments, J. Biol. Chem. **169**:583-594, 1947.

15. Bale, W. F., and Spar, I. L.: In Vivo Localization of Rat Organ Antibodies in Ovaries, Adrenals, and Other Tissues, J. Immunol. **73**:125-133, 1954.

16. MacNider, W. deB.: Morphological Basis for Certain Tissue Resistance, Science **73**:103-105, 1931.

17. Conn, H. L., Jr.; Wilds, L.; Helwig, J., and Ibach, P.: Study of the Renal Circulation, Tubular Function and Morphology, and Urinary Volume and Composition in Dogs Following Mercury Poisoning and Transfusion of Human Blood, J. Clin. Invest. **33**:732-741, 1954.

18. Oliver, J.; MacDowell, M., and Tracy, A.: Pathogenesis of Acute Renal Failure Associated with Traumatic and Toxic Injury: Renal Ischemia, Nephrotoxic Damage and the Ischemuric Episode, J. Clin. Invest. **30**:1305-1440, 1951.

19. MacNider, W. deB.: Acute Experimental Nephritis, Physiol. Rev. **4**:595-638, 1924.

20. Taylor, H. L.; Mickelsen, O., and Keys, A.: Effects of Induced Malaria, Acute Starvation and Semi-Starvation on the Electrophoretic Diagram of the Serum Proteins of Normal Young Men, J. Clin. Invest. **28**:273-281, 1949.

21. Greenspan, E. M.; Lehman, I.; Graff, M. M., and Schoenbach, E. B.: Comparative Study of the Serum Glycoproteins in Patients with Parenchymatous Hepatic Disease or Metastatic Neoplasia, Cancer **4**:972-983, 1951.

22. Gérard, P.: Comparative Histophysiology of Vertebrate Nephron, J. Anat. **70**:354-379, 1936.

23. Paldino, R. L., and Hyman, C.: Mechanism Whereby Renin Increases the Rate of T-1824 Disappearance from the Circulation of Rabbits, Am. J. Physiol. **179**:599-600, 1954.

24. Rather, L. J., and Addis, T.: Renin Proteinuria in the Rat, J. Exper. Med. **91**:567-572, 1950.

25. Brandt, J. L., and Gruhn, J. G.: Effect of Renin on Proteinuria and PAH Clearance at Low Plasma Levels, Am. J. Physiol. **153**:458-464, 1948.

26. Lippman, R. W.; Ureen, H. J., and Oliver, J.: Mechanism of Proteinuria: Effect of Renin on Hemoglobin Excretion, J. Exper. Med. **93**:605-614, 1951.

27. Spector, W. G.: Reabsorption of Labeled Proteins by the Normal and Nephrotic Rat Kidney, J. Path. & Bact. **68**:187-196, 1954.

Pulmonary Microlithiasis

Microlithiasis Alveolaris Pulmonum

G. KENT, M.D.
E. S. GILBERT, M.D.
and
H. H. MEYER, M.D., Chicago

The following report deals with a rare form of pulmonary calcification, named "microlithiasis alveolaris pulmonum" by Puhr [1] in 1933. The essential feature of the condition is a striking radiographic opacity resembling miliary tuberculosis, in contrast to almost complete absence of symptoms. The radiologic appearance is caused by innumerable intra-alveolar "calculi" throughout the lung. The first case report dates back to 1856 * and was reported under the name of "corpora amylacea." Since that time we have been able to find a total of 14 cases,† all in the European literature.

REPORT OF A CASE

Clinical History

The clinical aspect of this condition will be discussed elsewhere, and so only a summary of the clinical findings will be given here.

A 35-year-old white man, born in Dallas, who otherwise was perfectly well, was hospitalized repeatedly because of excessive alcoholic intake. During one of these bouts, in the spring of 1954, on routine chest roentgenologic examination (Fig. 1) the diagnosis of "miliary tuberculosis" was made, and he was transferred to a sanitarium. There he received combined chemotherapy but left after four months. A field nurse saw him a few months later, and he was admitted to the Municipal Tuberculosis Sanitarium, Chicago, in February, 1955.

Received for publication August 13, 1955.

From the Municipal Tuberculosis Sanitarium and Department of Pathology, Cook County Hospital.

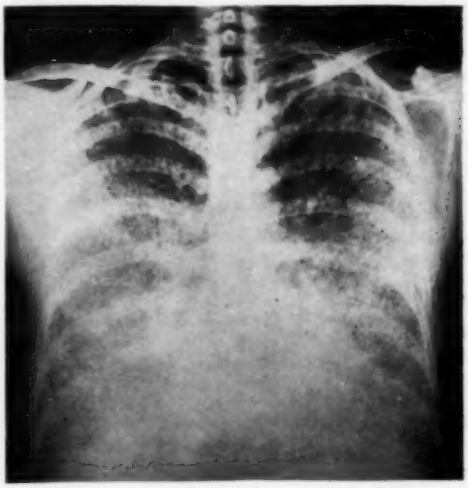
* References 2 and 3.

† References 1 through 12.

The patient had had measles during childhood and a chancre in 1935, which was inadequately treated. He worked as a cotton picker from 1931-1937, and from 1937-1942 he worked with a fertilizer company, where he handled sulfur rocks, which were later ground and mixed with "some kind of earthy material." In this work he had to wear a mask because of the high dust concentration. After two years of unemployment he worked in a rug factory for two years and was exposed to cotton and wool dust. After 1946 he had worked in restaurants until the time of admission.

Physical findings were negative. Serologic study for syphilis yielded doubtful results. The spinal fluid protein was slightly elevated (84 mg. per 100 cc.). The tuberculin test was positive, as was the histoplasmin test (1:500). Serum calcium and phosphorus were normal. Roentgenologic study of the skeleton, bacteriologic studies, and biopsies of the liver, scalene node, and bone marrow yielded negative results. On biopsy, the lung was found to be practically free of adhesions. On palpation of the right upper and middle lobes, there was a diffuse granular and gritty feel. A specimen 2×2×1 cm. was obtained.

Fig. 1.—Roentgenogram, showing diffuseness of lesion with greater density toward the bases.



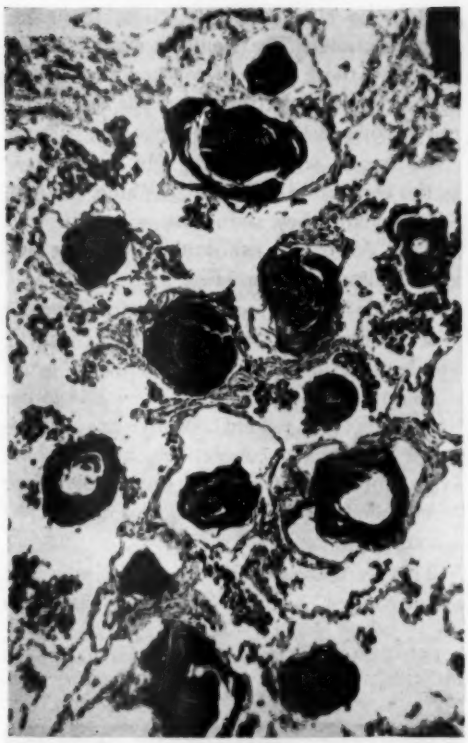


Fig. 2.—Calcospherites, distribution of mononuclear cells, and minimal changes of alveolar septa; reduced about ¼ from mag. × 50.

Pathologic Observation

On section of the specimen the normal pulmonary architecture was obscured by innumerable barely visible gray or yellow nodules. Decalcification was necessary before paraffin embedding could be done.

Approximately one-third of the alveoli contain concretions morphologically similar to corpora amylacea, ranging from 0.02 to 0.3 mm. in diameter (Fig. 2). The distribution is focally accentuated. The concretions show either concentric lamination, often with radial striation, or "onion skin" lamination which may become very irregular. Others represent homogeneous structureless masses. The lamellae may be seen along the entire circumference, or they may form crescents or irregular masses. Many concrements have splits through numerous planes, probably due to shrinkage during preparation. They usually occupy only a portion of the alveolar spaces. However, in several areas it is apparent that they had once occupied the entire space. Slender portions of the concretions are still in close alignment with the alveolar wall, and the elastic stain also shows fibers in the alveolar wall which enter the alveolar space and continue in the periphery of the concretions. The layers vary in thickness and seem arranged around a central portion, the size, structure, and staining properties of which show great variation; it contains no pigment or fibers and is either granular or homogeneous. The concretions usually occur singly within an alveolus, but occasionally several are seen within an alveolus or one may be found in a bronchiole (Fig. 3). In the periphery of some of the concrements, or occasionally within a concrement, irregular bony lamellae are found (Fig. 4).

The staining properties of the lamellae and the central portions vary in hematoxylin and eosin stains. The central portion may

Fig. 3.—Concretion within bronchiole; reduced about ¼ from mag. × 75.

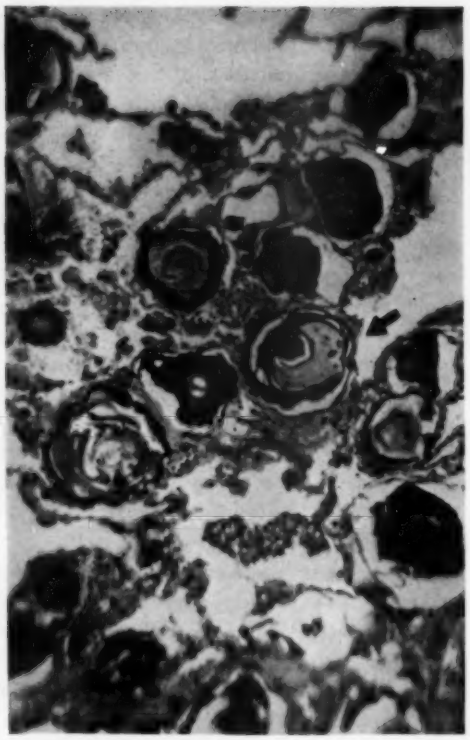


Fig. 4.—Ossification within calcospherite (arrow); reduced about ¼ from mag. × 50.

stain lighter or darker than the periphery, the shades varying from pink or red to violet or blue. With the Van Gieson stain there is a fairly homogeneous yellow color, except for the bony lamellae, which stain red. The von Kossa stain in the untreated specimen is strongly positive. Metachromatic, Congo red, and iodine stains for amyloid are negative. With iodine a yellow color is produced which does not change after treatment with 10% sulfuric acid. A similar reaction is obtained when the tissue is previously treated with sulfuric acid for 24 hours.

The concretions stain fairly intensely with the periodic acid-Schiff reagent and are positive with the colloidal-iron stain. They appear to have a greater affinity for the colloidal-iron stain than for the PAS, when the two stains are applied concurrently by use of the Rinehart[18] technique.‡ They contain no elastic fibers or iron (Prussian blue reaction). The Feulgen stain is negative, and there is no pyroninophilia; nor is there optical activity under the polarizing microscope in the paraffin-embedded tissues.

In frozen sections irregular small areas in the concretions are Sudanophilic (brownish red) and they stain blue with Nile blue sulfate. All bodies are doubly refractile and give Maltese crosses (Fig. 5).

Outside of the concretions the alveolar spaces either are free or contain varying numbers of mononuclear cells. These occur in clumps and occasionally show an epithelium-like arrangement. Many are aggregated around the concretions, and in some areas multinucleated giant cells, resembling foreign-body giant cells, are encountered among them.

The alveolar walls show surprisingly few abnormalities. Several concretions are seen

Fig. 5.—Frozen section showing Maltese cross under polarizing light; reduced about ¼ from mag. × 50.

‡ Done through the courtesy of Dr. R. W. Wissler, University of Chicago.

within alveoli, the walls of which are entirely normal. However, other septa show slight fibrous thickening (Fig. 6), and fibrosis is more frequent in those alveoli that contain concretions. The elastic network is fairly well preserved, although in several areas there is condensation or breaking-up of the fibers. Only rarely do the septal cells become prominent and assume a cuboidal lining. The interstitial connective tissue is only slightly thickened.

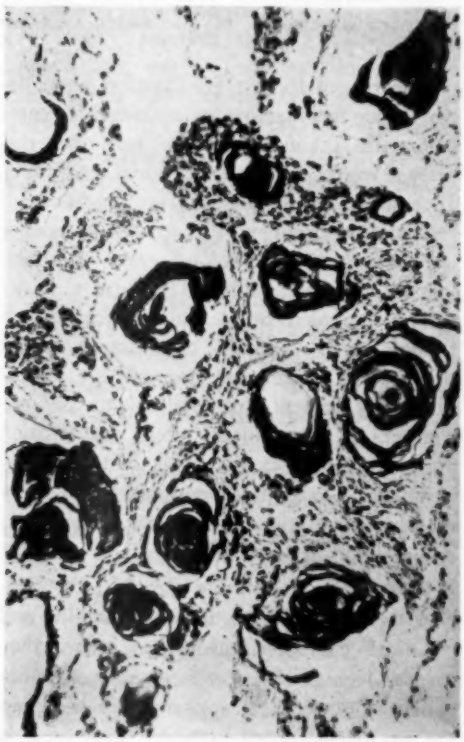


Fig. 6.—Fibrous thickening of alveolar septa; reduced about ¼ from mag. × 50.

COMMENT

When analyzing the 14 reported cases § and adding our own in search of a common etiologic agent, one encounters a variety of factors which are not easy to interpret. In four cases ‖ there was a history of a single or repeated attacks of pneumonia, and in two cases ¶ the chief associated condition was mitral stenosis. Four other cases,# while not showing associated diseases, revealed exposure to "dust"; two of these patients were masons, one was a rock driller, and one was exposed to clay dust. Our case also falls into this category. In three cases * there was no evidence of associated disease or occupational hazard. It may be pertinent, however, that, overlapping with the above, seven † of the total group were engaged in farm work for a significant time. The ages range from 8 to 72, the majority being around 40; the sexes are about equally represented. In one case report [11] the disease is described in a sister, her brother, and the latter's son.

In view of the morphologic resemblance of the concretions to corpora amylacea of the lung, the pertinent features of the latter are briefly summarized. Corpora amylacea or amyloidea are laminated structures, usually concentrically arranged around a central core giving positive reactions to amyloid stains. In the lung they occur with greatest frequency in emphysema, chronic bronchitis, congestion, pneumonias, infarctions, and atelectasis,[14] conditions which presumably provide opportunities for the formation and stagnation of intra-alveolar material. Lubarsch and Plenge [14] in a detailed survey conclude that (1) corpora amylacea are formed directly or indirectly through changes in the septal cells; (2) they are relatively bland and have little effect on the alveolar walls; a slight irritational effect, however, as indicated by the presence of foreign-body giant cells, is not denied; (3) in view of certain differences in staining reactions the material in corpora amylacea is not identical with amyloid. In their own studies the bodies contained no collagen or elastic fibers, and in frozen sections they were Sudanophilic but not doubly refractile. Steel and coworkers [15] showed that corpora amylacea

§ References 1 through 12.

‖ References 1, 10, and 11.

¶ References 2, 3, and 9.

References 5, 7, 8, and 12.

* References 4, 6, and 11.

† References 5, 6, 11, and 12.

contain polysaccharides but no nucleic acids, either in the form of deoxyribose or ribose nucleic acids. They conclude that corpora amylacea are formed from inspissated secretion, and that there is no evidence of cells as a constituent.

The concretions in this case show the following differences from corpora amylacea usually described: (1) The amyloid stains are uniformly negative; (2) they contain doubly refractile lipids; (3) structurally they are more irregular; (4) they are diffuse, and unlike corpora amylacea they occur in areas not apparently the seat of previous pathologic change; (5) they are heavily calcified.

The first four of these differences are compatible with unusual variants of corpora amylacea, while calcification of corpora amylacea may occur in the prostate [16] and lung.‡ The concretions then may merely represent calcified corpora amylacea, or at least be formed by a fundamentally similar process. The morphology of the concretions, the cellular reaction, as well as the heterogeneous etiologic factors would seem to support this view.

On the other hand, the enumerated etiologic factors might only represent the basis for "stasis," or be entirely coincidental, and a more specific factor, common to all, might be responsible. The diffuse character of the lesion, the variation in size and age, and the laminated appearance suggest the presence of a general stimulus occurring in successive stages. This may have been in the form of a hyperimmune or other peculiar reaction to circulating or inhaled irritants, or in the form of a bacterial, protozoal, or mycotic infection. The reported occurrence of this disease in a family [11] can be interpreted either way. Manz [13] recently described a case in which threads of calcified material were interpreted as calcified fungi. No evidence for any of the above-mentioned factors was obtained in our case, although the histoplasmin test was positive. Morphologically, the intra-alveolar location of the bodies bears no resemblance to the calcific changes found in histoplasmosis.

So far as terminology is concerned, the tissue removed at operation was not sufficient for chemical analysis. Judging from the literature,§ however, the calcium phosphate and carbonate content of the concretions relative to organic matrix, where examined, would seem to warrant the term calculus. The name "microlithiasis alveolaris pulmonum" given to the condition by Puhr would therefore seem preferable to that of "corpora amylacea" of the older German literature.

The reason for the calcification must depend upon local factors, as nothing implicates metastatic calcification. The rarity of this disease would suggest exceptional conditions. In analogy, infrequent to rare instances of calcification occur in the stroma of adenocarcinomas of the colon [17] and in malakoplakia of the bladder. The former shows infrequent calcification and ossification, although such suitable conditions as the presence of mucin and necrosis are almost invariably present. In malakoplakia one encounters a mononuclear cell proliferation and intra- and extracellular concentrically laminated calcospherites reminiscent of pneumoliths. Again, the disease is not etiologically specific and is associated with urostasis.[18]

Tissues involved in dystrophic calcification are of widely different origin and chemical character. Morphologically, they seem to have in common the quality of homogeneity and physical appearance resembling the hyaline cartilage in which new bone is formed.[19] Chemically, some of the factors to be borne in mind are reduction of the tissue concentration of hydrogen ions; electronegative charge of dead or injured tissue [20]; enzymatic processes such as phosphorylative glycolysis and presence of phosphatase; as well as nonenzymatic processes [21] such as the ion-binding capacity, through their acid groups, of acid mucopolysaccharides, particularly chondroitin sul-

‡ References 2 and 3.

§ References 6, 9, and 12.

furic acid.[22] In our case the concretions were not metachromatic; however, they were periodic acid-Schiff-positive and interestingly enough had a strong affinity for the colloidal-iron stain.

Of the etiologic factors associated with pulmonary alveolar microlithiasis, rheumatic heart disease seems of particular interest. Mitral stenosis may be associated on rare occasions with disseminated calcification and ossification of the lung.‖ The fluid which exudes in rheumatic pneumonitis is rich in protein and frequently forms a thick eosinophilic lining referred to as hyaline membrane.[27] The peculiar quality of the exudate, which is not easily reabsorbed, would seem to lend itself to early organization and occasional calcification or ossification. Elkeles and Glynn [26] describe bony nodules situated within alveolar ducts and alveoli and suggest that they are the late result of specific rheumatic pneumonia, with bone formation taking place in the alveolar exudate. These nodules are not the result of long-standing passive congestion, since cases have been observed in which no clinical or radiological evidence of pulmonary congestion was present.[26] Moreover, no cases of osseous nodules in the lungs have been reported in chronic passive congestion due to causes other than rheumatic valvular disease.[26] While rheumatic pneumonitis would therefore appear to be implicated, it must be remembered that the morphologic changes of this disease in the lung are not specific.¶

From the evidence at present available we would regard pulmonary microlithiasis to be the result of a peculiar exudative response to a variety of insults.

Judging from the literature, the disease is slowly progressive, the longest observation period being 25 years.[12] In most reports the changes found at autopsy are complicated by associated conditions, such as mitral stenosis or pulmonary fibrosis; in our case there has been a relationship between fibrosis and alveoli containing concretions. The progression is explained by a low-grade irritational effect and/or recurrent activity of the underlying process.

‖ References 23 through 26.

¶ References 27 and 28.

SUMMARY

A case of pulmonary microlithiasis (microlithiasis alveolaris pulmonum) is reported.

Etiological considerations based on 14 previous case reports are not conclusive. The disease is thought to be the result of a peculiar exudative response to a variety of insults.

REFERENCES

1. Puhr, L.: Mikrolithiasis alveolaris pulmonum, Arch. path. Anat. **290**:156-160, 1933.

2. Friedreich, N.: Corpora amylacea in den Lungen, Arch. path. Anat. **9**:613-618, 1856.

3. Friedreich, N.: Weitere Mitteilung über Corpora amylacea in den Lungen, Arch. path. Anat. **10**:201-219, 1856.

4. Schildknecht, O.: Zur Pathogenese verkalkter Schichtungskugeln sog. "Corpora amylacea," in der Lunge (unter Mitteilung eines ungewöhnlichen Falles), Arch. path. Anat. **285**:466-480, 1932.

5. Mariani, B.; Montanini, N., and Tortelli, G.: Microlitiasi polmonare endoalveolare diffusa, Ann. Ist. Carlo Forlanini **10**:179-199, 1947.

6. Leicher, F.: Über eine generalisierte Lungenerkrankung mit Konkrementbildung (Mikrolithiasis alveolaris pulmonum), Zentralbl. allg. Path. **85**: 49-62, 1949.

7. Bénard, H.; Rambert, P.; Pequignot, H.; Tissier, and Galistin, P.: Microlithiase alvéolaire diffuse, Bull. et mém. Soc. méd. hôp. Paris **66**: 482-485, 1950.

8. Lindig, W.: Ein klinisch-röntgenologischer Beitrag zum Krankheitsbild der "Mikrolithiasis alveolaris pulmonum," Fortschr. Geb. Röntgenstrahlen **75**:678-684, 1951.

9. Sharp, M. E., and Danino, E. A.: Unusual Form of Pulmonary Calcification: Microlithiasis Alveolaris Pulmonum, J. Path. & Bact. **65**:389-399, 1953.

10. Petrányi, G., and Zsebök, Z.: Mikrolithiasis alveolaris miliaris pulmonum, Radiol. clin. (Basel) **23**:202-209, 1954.

11. Mikhailov, V.: Pulmolithiasis endoalveolaris et interstitialis diffusa, Klin. med. **32**:31-36, 1954.

12. Manz, A.: Mikrolithiasis der Lungen mit Pilzbefall, Beitr. Klin. Tuberk. **111**:598-606, 1954.

13. Rinehart, J. F., and Abul-Haj, S. K.: Improved Method for Histologic Demonstration of Acid Mucopolysaccharides in Tissue, A. M. A. Arch. Path. **52**:189-194, 1951.

14. Lubarsch, O., and Plenge, K.: Die Corpora amylacea, in Henke, F., and Lubarsch, O., editors: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Springer-Verlag, 1931, p. 617.

15. Steele, H. D.; Kinley, G.; Leuchtenberger, C., and Lieb, E.: Polysaccharide Nature of Corpora Amylacea, A. M. A. Arch. Path. **54**:94-97, 1952.

16. Maximow, A. A., and Bloom, W. A.: A Textbook of Histology, Philadelphia & London, W. B. Saunders Company, 1948, p. 534.

17. Van Patter, H. T., and Whittick, J. W.: Heterotropic Ossification in Intestinal Neoplasm, Am. J. Path. **31**:73-91, 1955.

18. Bleisch, V. R., and Konikov, N. F.: Malakoplakia of Urinary Bladder, A. M. A. Arch. Path. **54**:388-397, 1952.

19. Barr, D. P.: Pathological Calcification, Physiol. Rev. **12**:593-624, 1932.

20. Mulligan, R. M.: Metastatic Calcification, Arch. Path. **43**:177-230, 1947.

21. McElroy, W. D., and Glass, H. B.: Phosphorus Metabolism, Baltimore, The Johns Hopkins Press, 1952, Vol. 2.

22. Sobel, A. E.: Studies on the "Local Factor" of Calcification, in Reifenstein, E. C., Jr., and Wells, S. L., editors: Metabolic Interrelations with Special Reference to Calcium, Transactions of Fourth Conference, Jan. 7-8, 1952, New York, Josiah Macy, Jr. Foundation, 1952.

23. Jona, G.: Stenosi mitralica a forma emoftoica Induramento bruno del pulmone, calcificante, Policlinico (sez. prat.) **40**:1679-1685, 1933.

24. Lawson, H. M.: Disseminated Ossification of Lungs in Association with Mitral Stenosis, Brit. M. J. **1**:433-434, 1949.

25. Munk, E.: Calcifications multiples disséminées dans les poumons dans la maladie mitrale, J. radiol. et électrol. **23**:58-62, 1939.

26. Elkeles, A., and Glynn, L. E.: Disseminated Parenchymatous Ossification in Lungs in Association with Mitral Stenosis, J. Path. & Bact. **58**:517-522, 1946.

27. Kuzma, J. F., and Lustok, M. J.: Rheumatic Fever Pneumonitis, Am. J. Path. **27**:696-697, 1951.

28. Epstein, E. Z., and Greenspan, E. B.: Rheumatic Pneumonia, Arch. Int. Med. **68**:1074-1094, 1941.

News and Comment

PERSONAL NEWS

Retirement of Dr. Frieda S. Robbins.—Dr. Frieda S. Robbins has retired as Associate in Pathology at the University of Rochester School of Medicine and Dentistry after 32 years of service.

Dr. Harry M. Zimmerman in Israel and London.—Dr. Harry M. Zimmerman, Chief, Laboratory Division, Montefiore Hospital, New York, recently was in Israel as a consultant in relation to the organization of the Hadassah-Hebrew University Medical Center. He also presented a paper on brain tumors before the International Congress of Neuropathology in London, England.

Ward Burdick Award to Dr. George Gomori.—Dr. George Gomori of the Department of Medicine, University of Chicago, was the recipient of the Ward Burdick Award for 1955, given by the American Society of Clinical Pathologists at their annual meeting in Chicago, on Oct. 11, 1955.

Toxoplasmosis of Newborn

Histologic Changes in Mother's Lymph Node with Presumptive Isolation of Toxoplasma from Mother's Lymph Node by Mouse Passage

CARTER M. ALEXANDER, M.D., Covina, Calif.
and
JOHN W. CALLISTER, M.D., Pasadena, Calif.

The primary purpose of this paper is to present the morphologic changes in a cervical lymph node of a woman whose autopsied newborn infant was proved to have died of congenital toxoplasmosis; also, organisms consistent with Toxoplasma gondii were found in a mouse injected with tissue from that lymph node.

Toxoplasma was the name given to a new species of Protozoa that Nicolle and Manceaux observed, in 1908, in a North African rodent, the gondi.[1] Many subsequent reports have shown the parasite to be widely distributed in nature. Summarizing, Jacobs [2] states that Toxoplasma has been found, among other hosts, in the gondi, mouse, vole, rat, guinea pig, squirrel, marmot, dog, cat, chinchilla, sheep, baboon, chimpanzee, man, and pigeon. He believes that there is no reason to doubt that infection is widespread in other birds. He states that reports of Toxoplasma in cold-blooded animals (reptiles), based solely on morphologic grounds, require biologic confirmation. The parasite is world-wide in distribution.

The mode of transmission of the disease, from animal to animal and from animal to man, is unproved. However, Jacobs, analyzing present information, recognizes the possibility that carnivores may acquire toxoplasmosis by consuming infected tissue, and that possible transmission by arthropods (ticks) should be investigated further.

The human syndrome of congenital toxoplasmosis, which may include neonatal meningoencephalitis, hydrocephaly, chorioretinitis, and cerebral calcifications, was first described in 1937 by Wolf and Cowen,[3] with proof of Toxoplasma as the causative organism by animal transmission demonstrated by Wolf, Cowen, and Paige in 1940.[4] In the monograph of Frenkel and Friedlander,[5] there are excellent descriptions of the pathology, pathogenesis, diagnosis, and treatment of neonatal toxoplasmosis.

The clinical manifestations of human toxoplasmosis are summarized by Feldman,[6] who noted that there are congenital and acquired types. While most acquired infections are clinically inapparent, proved acquired toxoplasmosis has been manifested by fever, maculopapular rash simulating the typhus-spotted-fever group, by encephalitis, myalgia, arthralgia, myocarditis, lymphadenitis, cough, pneumonitis, and possibly conjunctivitis. In human adults, European investigators have described a syndrome simulating infectious mononucleosis, but with negative heterophile antibody tests, characterized by fever, lymphadenopathy, lymphocytosis, and abnormal lymphocytes in the peripheral blood.*

Received for publication Aug. 8, 1955.

Read in part before the Section on Pathology and Bacteriology at the 84th Annual Session of the California Medical Association, San Francisco, May 1, 1955.

From the Department of Pathology, Collis P. and Howard Huntington Memorial Hospital, Pasadena. Formerly Pathologist, Collis P. and Howard Huntington Memorial Hospital, Pasadena; present address: Pathologist, Covina Inter-Community Hospital (Dr. Alexander). Resident in Pathology, Collis P. and Howard Huntington Memorial Hospital (Dr. Callister).

* References 7 and 8.

For the future control of the human congenital disease, it should be of interest to determine the absence of symptoms or the date of onset and nature of the symptoms in the mother, as well as whether the maternal disease is a generalized infection (as is probable) or localized to the genital tract. Toxoplasma has been isolated from a uterine curettage specimen and from the placenta,[9] and in two other instances Toxoplasma was found microscopically in the placenta.†

As for Toxoplasma involving human adult lymph nodes, we have found the microscopic details described once [8]; organisms considered to be Toxoplasma in stained microscopic sections have been reported three times,‡ and Toxoplasma isolated from lymph nodes by animal injection, twice.§

REPORT OF A CASE

A 21-year-old white primipara, native of the Los Angeles area, was delivered on Feb. 24, 1953, after 32 weeks' gestation, of a boy weighing 3000 gm. and 43 cm. in height who lived 45 minutes. The mother had abdominal cramps and vaginal bleeding intermittently for 2 months before delivery, although afebrile, and had spontaneous rupture of the membranes 11 days before onset of spontaneous labor. Close questioning elicited the fact that she developed painless cervical lymphadenopathy, without other associated symptoms, after six months of pregnancy. Her blood Wassermann and Kahn reactions were negative. In September, 1951, she had been treated by her local physician for "an infection of Skene's glands."

At autopsy of the infant, by one of us (J. W. C.), five hours after death, there was marked generalized edema and cyanosis, with marked scalp edema giving the ears the appearance of abnormally low location. The fontanels were normal-sized; there was no overlapping of the cranial bones or widening of the cranial sutures. Subarachnoid fluid was scanty, the brain being tightly applied, although not adherent, to the dura. Swabs from the ventricular exudate showed no organisms on Gram or Wright's stain, and culture anaerobically showed no growth after 48 hours.

After formalin fixation, the brain weighed 103 gm. after collapse and drainage of cloudy yellow ventricular fluid. Both cerebral hemispheres were profoundly enlarged, each estimated to measure 9.5×6.5×5-6 cm. Their walls were markedly thinned. The external surfaces showed marked ironing-out, and only occasionally could one see residual folds or convolutions. Blood vessels in the leptomeninges were everywhere widely engorged, with moderate brown staining of the meninges. Parts of both hemispheres were represented by thin zones of completely necrotic, yellow-white, mushy tissue only 1 to 3 mm. thick, easily scraped away by the finger, leaving only the meninges. Other, apparently viable, portions were as much as 7 mm. thick. Much of the ependymal surface was quite rough, dull, and lusterless, owing to yellow exudate on its surface. The basal ganglia were fairly large and not compressed to any noteworthy degree. Both ganglia had large irregularly shaped areas of yellow necrosis. The markedly dilated third ventricle measured fully 2×2×1.8 cm., with a smooth glistening ependymal lining. Owing to artifactual tearing, the foramina of Monro could not be seen. The aqueduct of Sylvius was patent throughout, up to 1 mm. in diameter. There was moderate dilatation of the fourth ventricle, the ependymal surface of which was smooth, with flattening of the ventral surface. Moderate thickening of the leptomeninges by fibrous tissue was found over the lateral surfaces of the pons and medulla and over the greater portion of the cerebellum. Multiple sections showed apparently viable tissue in the pons and medulla, but large portions of the cerebellum, particularly portions beneath the meninges, were necrotic, with moderate cerebellar atrophy.

Gross examination of the torso showed no serous effusions. The pink-tan thymus measured only 4×3×3 mm. The incompletely expanded lungs together weighed 20 gm., showing small pleural hemorrhages, pink meaty parenchyma that sank in water, and an empty tracheobronchial tree. The normal-sized heart weighed 16 gm. and was without anomaly or other gross change. The rather large liver weighed 145 gm., and the soft red-tan parenchyma had a visible lobular pattern. Biliary tract was normal. The large spleen weighed 40 gm. and had firm homogeneous dark red pulp. The pancreas, adrenal glands, kidneys, and alimentary tract were grossly normal. Lines of ossification of the femurs at the knees were straight and narrow.

The placenta was somewhat enlarged and weighed 650 gm. The fetal surface was smooth, gray-white, and glistening. Membranes were not thickened. The cotyledons were torn, unusually firm, and pink-tan, without infarcts.

Microscopic examination showed, in sections of cerebral hemispheres, massive coagulation necrosis of cortical tissue extending in

† Reference 10 and Neghme,[11] cited in reference 10.

‡ References 12 and 13 and Pinkerton, H., cited by Sabin.[17]

§ References 14 and 15.

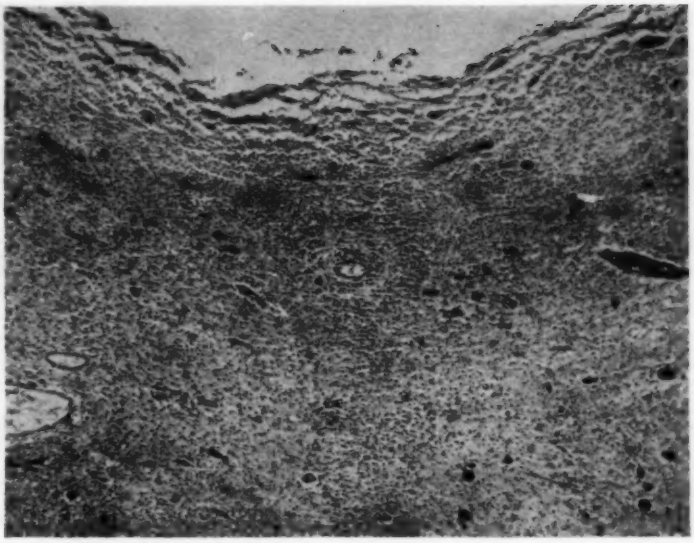


Fig 1.—Infant's cerebrum: necrotic ependymal lining. Hematoxylin and eosin; reduced slightly from mag. × 60.

places from the necrotic ependymal lining to the pia-arachnoid (Fig. 1). Necrotic tissue was dusted with nuclear debris, but here the tissue pattern was fairly well-preserved. Many small blood vessels contained fibrinous thrombi, and a curious change was the presence of small vessels with necrotic walls whose lumens contained rather well-preserved red blood cells (Fig. 2). In the subarachnoid space there was heavy infiltration by histiocytes, with a few plasma cells and neutrophiles. Where a thin rim of viable cortical gray matter remained, it was edematous and infiltrated by neutrophiles. At the margins of necrotic areas, viable tissue contained occasional glial nodules, composed of closely clustered rod-shaped nuclei, sometimes mingled with a few neutrophiles (Fig. 3). With fair ease one could find, in viable tissue near necrotic areas, large mononuclear cells (presumably phagocytic) whose cytoplasm contained groups of 8 to 10 or more parasites (Fig. 4). These

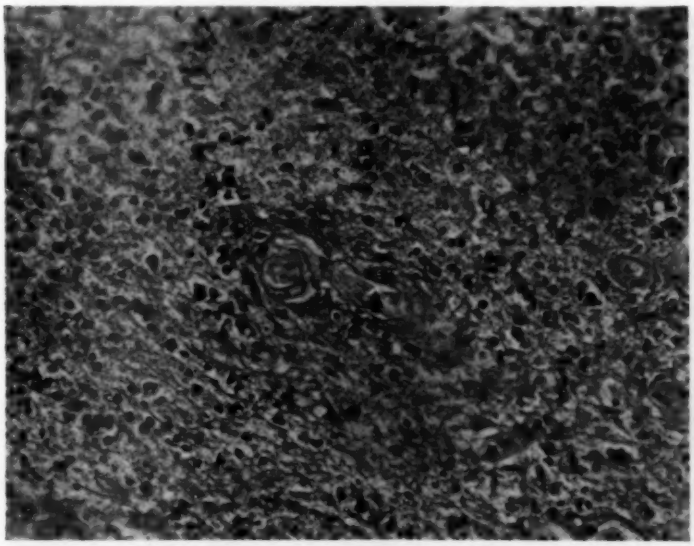


Fig. 2.—Infant's cerebrum: necrotic tissue with thrombosed necrotic vessels. Hematoxylin and eosin; reduced slightly from mag. × 285.

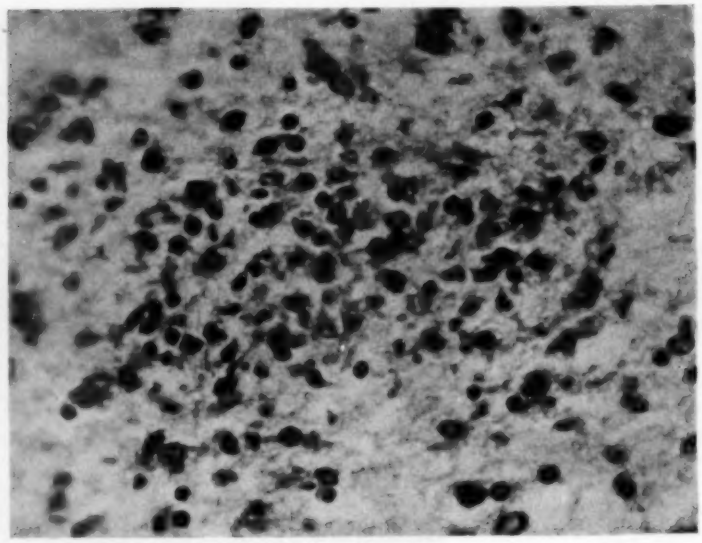


Fig. 3.—Infant's cerebrum: glial nodule. Hematoxylin and eosin; reduced slightly from mag. × 520.

were easily found after hematoxylin and eosin or after Giemsa staining. (The Hotchkiss-McManus stain was used only on some visceral sections, with negative results.) In sections, the organisms were about one-third the diameter of a red blood cell, rounded or oval, with pale acidophilic cytoplasm and a single pale-staining, but definite, nucleus. Parasites may have been more numerous in necrotic zones, where we could not certainly distinguish them from nuclear debris, but in viable tissue we could find occasional single extracellular parasites and occasional small groups of parasites in cells that had lost their nuclei. One cystic structure, larger in size than a mononuclear phagocytic cell, contained perhaps 50 parasites, with free parasites in adjacent tissue. Sections of both basal ganglia showed profound necrosis, covered sometimes only by thin rims of edematous cortical gray matter. In the ventricular lining there were small areas of intact ependyma with

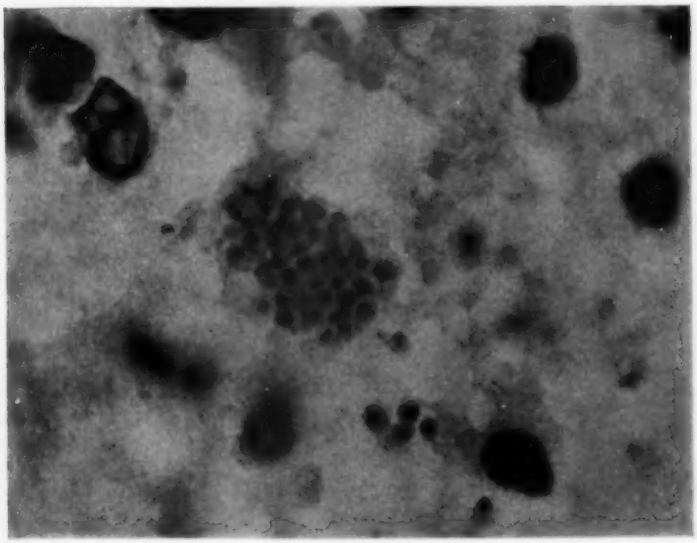


Fig. 4.—Infant's cerebrum: intracellular Toxoplasma proliferative forms. Hematoxylin and eosin; reduced slightly from mag. × 1600.

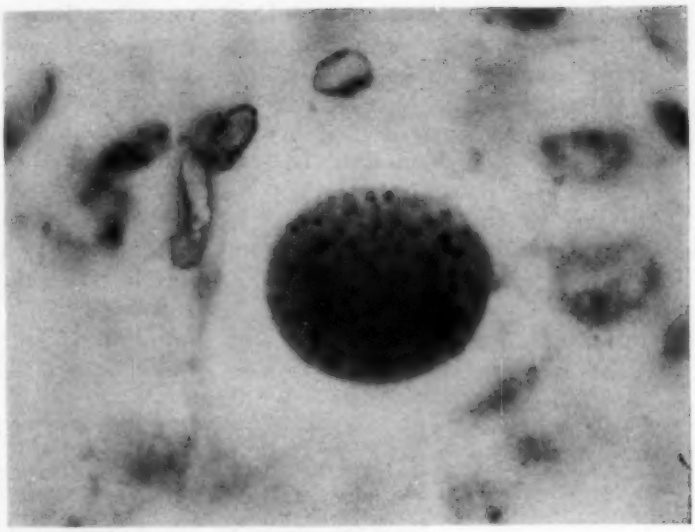


Fig. 5.—Infant's pons: Toxoplasma pseudocyst. Hematoxylin and eosin; reduced slightly from mag. × 1600.

well-preserved brain tissue. There was necrosis of tissue at the root of the choroid plexus; the plexus was edematous, and its stroma was infiltrated by mononuclear cells. In the midbrain the aqueduct was patent. A few microglial nodules were present. In the pons, there were microglial nodules and there was a single pseudocyst with a hyaline envelope, containing innumerable parasites (Fig. 5). The fourth ventricular ependyma was well preserved. There was massive necrosis of portions of the cerebellum, without the finding of parasites. The medulla contained only a few glial nodules. We found parasites only in the brain.

In a midsagittal section of an eye, a large portion of the retina was detached and pushed anteriorly, and the space so formed was filled with loose edematous connective tissue. There were areas of necrosis, dusted by nuclear fragments, involving large portions of the choroid and in places involving the retina. Newly formed vessels were present in the choroid. The lens was lost in the section.

Fig. 6.—Infant's eye: chorioretinitis; retinal detachment. Hematoxylin and eosin; reduced slightly from mag. × 80.

The anterior chamber contained fresh blood clot showing slight organization (Fig. 6).

In the heart there was interstitial infiltration by large numbers of histiocytes and lymphocytes, with a few neutrophiles and plasma cells, in both epicardium and myocardium, without muscle fiber necrosis. The lungs were free of inflammation and very incompletely expanded, with scales of vernix cells in a few alveoli. In the capillaries of thick alveolar septa there were numerous cells considered to be primitive erythroblasts, with large dark nuclei and thin rims of cytoplasm.

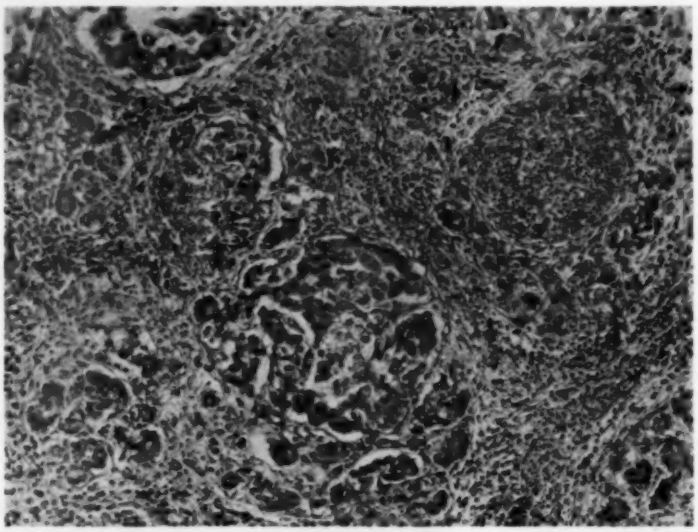


Fig. 7.—Infant's pancreas. Note islet necrosis. Hematoxylin and eosin; reduced slightly from mag. × 130.

In the thymus there were small foci of necrosis associated with neutrophiles; in the medulla there were numerous blood-forming cells, chiefly erythropoietic. In the liver the sinusoids were distended and literally filled by large groups of blood-forming cells, chiefly erythropoietic, and there were many large groups of primitive erythroblasts. Adjacent liver cells were somewhat attenuated, and some liver cells contained granular yellow pigment, but there was no notable hemosiderosis. In the spleen, follicles were practically absent and in the engorged red pulp pools of blood lay apparently between rigidly patent sinusoids. The sinusoids contained many clusters of blood-forming cells, mainly erythropoietic. Hemosiderosis was absent. In the pancreas there were profound changes: There was extensive interstitial fibrosis, with a heavy infiltration by cells that were chiefly hematopoietic. Acini were correspondingly shrunken, and ducts seemed normal. Nearly every islet of Langerhans showed partial or complete coagulation necrosis with formation of brightly acidophilic masses, apparently of necrotic islet cells. A few neutrophiles were seen here (Fig. 7). The adrenal glands had small hematopoietic foci in their inner cortices. Examination of the kidneys showed only a heavy cortical interstitial infiltration by cells that were mostly hematopoietic. The thyroid was normal. In all of the infant's organs, nucleated red blood cells were numerous within vessels. In the placenta, chorionic villi frequently exhibited a mild degree of hyalinization. In some villi the capillaries were displaced to the peripheries, but in other villi there were widely patent capillaries throughout. Nucleated red blood cells were present in rather higher numbers than normal.

Anatomic Diagnoses: Toxoplasmosis causing internal communicating hydrocephalus; profound necrosis of cerebral cortex, basal ganglia, and cerebellum; toxoplasmal chorioretinitis; interstitial pancreatitis with necrosis

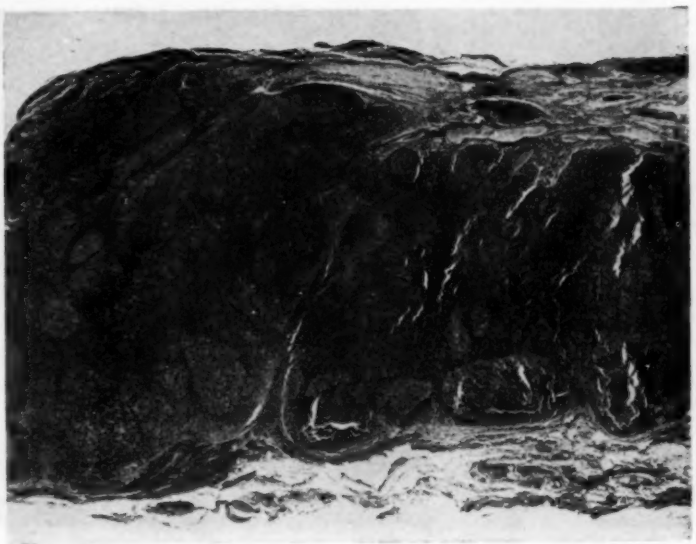


Fig. 8.—Mother's lymph node: follicular hyperplasia. Hematoxylin and eosin; reduced slightly from mag. × 18.

of islets of Langerhans; interstitial myocarditis; marked extramedullary hematopoiesis (liver, spleen, kidneys, pancreas, etc.); massive generalized edema.

POSTPARTUM STUDIES ON MOTHER

On April 11, 1953, about six and one-half weeks post partum, a right posterior cervical lymph node was excised; the node had been clinically manifest without change or symptoms since onset of lymphadenopathy, when the mother was six months' pregnant. The node was soft and red-tan and measured 2.1×1.8×1.2 cm. Half of the node was embedded after fixation in Zenker's fluid. Hematoxylin-and-eosin-stained sections showed fairly well-preserved architecture (Figs. 8 and 9). There were many enlarged cortical lymphoid follicles; their ill-defined large germinal centers had large pale primordial cells whose large nuclei showed numerous typical mitoses. These cells contained a few

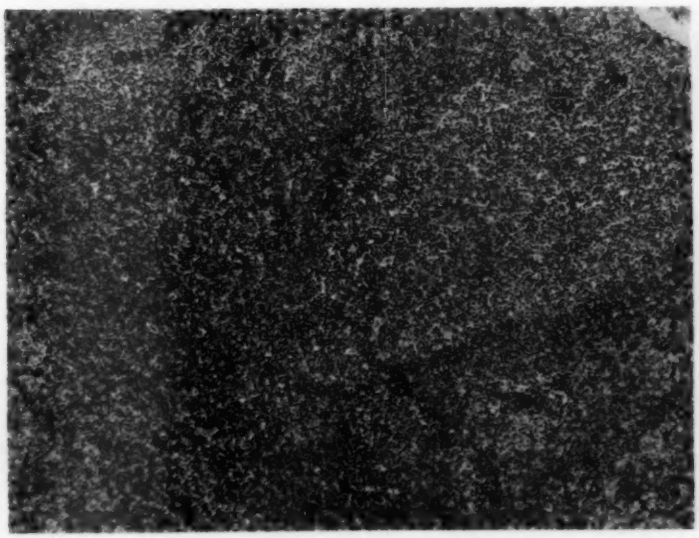


Fig. 9.—Mother's lymph node: follicle with large ill-defined germinal center. Hematoxylin and eosin; reduced slightly from mag. × 80.

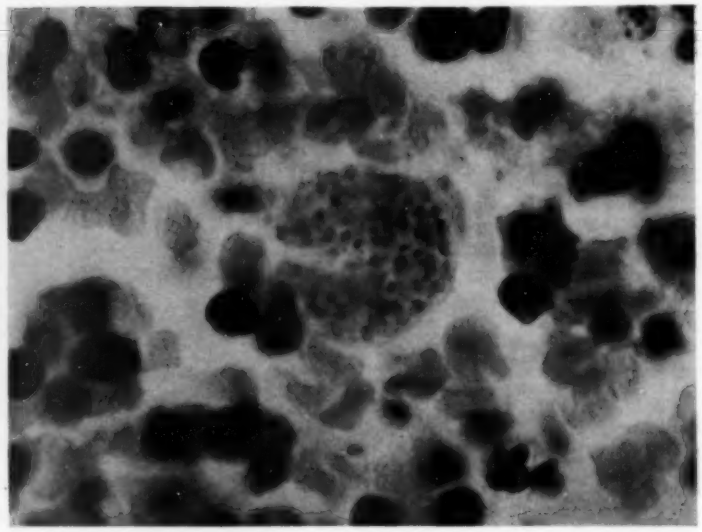


Fig. 10. — Mother's lymph node: Toxoplasma intracellular proliferative forms. Hematoxylin and eosin; reduced slightly from mag. × 1330.

tiny basophilic granules, considered to be products of karyorrhexis. The follicles tended to be distributed throughout the node, encroaching upon the medullary tissue. In the region of the peripheral sinus there were groups of large cells with pale cytoplasm and indented nuclei, of the histiocytic series. The medullary sinuses were narrow, somewhat obscured by scattered small- and medium-sized lymphocytes. The medullary cords contained scattered large mononuclear cells with small nuclei and abundant homogeneous acidophilic cytoplasm. The capsule was not infiltrated. After exhaustive study a single structure was found, in the region of the peripheral sinus, in a hematoxylin and eosin section. This structure appeared to be a cell containing intracellular proliferative Toxoplasma forms; there was no associated reaction or necrosis around the cell (Fig. 10). In a Giemsa-stained section, one other such parasitized cell was found (Fig. 11).

The remainder of the node was macerated without abrasives, emulsified in sterile saline

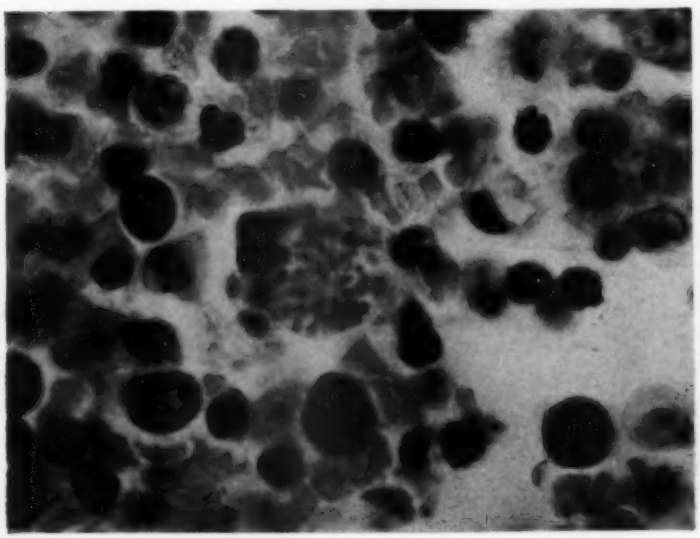


Fig. 11. — Mother's lymph node: Toxoplasma intracellular proliferative forms. Giemsa stain; reduced slightly from mag. × 1330.

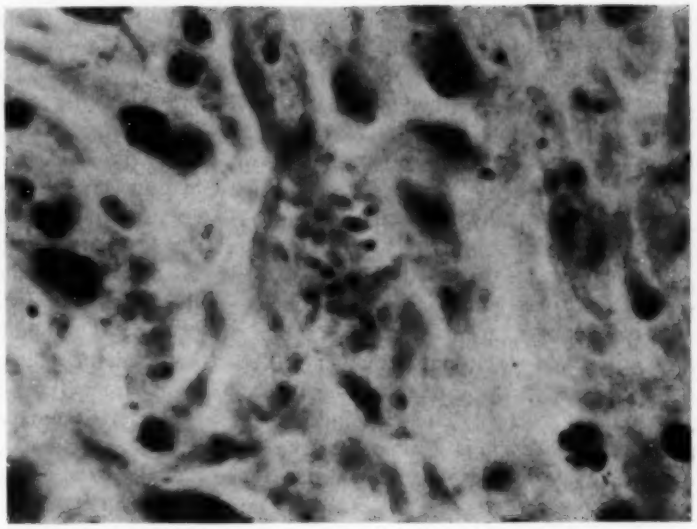


Fig. 12.—Mouse peripancreatic tissue: granuloma containing Toxoplasma. Giemsa stain; reduced slightly from mag. × 1600.

solution, and injected intraperitoneally into one three-month-old Swiss white mouse. Peritoneal aspiration on the fourth day yielded no fluid, and since the animal showed no outward signs of infection it was killed on the ninth day after injection. No gross changes in the organs were noted; however, in sections of the organs, a Giemsa-stained slide showed a small granuloma in peripancreatic tissue. In this lesion there were large mononuclear cells that contained groups of organisms that were consistent with Toxoplasma (Figs. 12 and 13).

Sections of the infant's organs, mother's lymph node, and mouse's organs were reviewed by Dr. J. K. Frenkel, who agreed that the organisms present were consistent with Toxoplasma and that the tissue reaction was compatible with that seen in toxoplasmosis.

On Oct. 19, 1953, a right posterior auricular lymph node, present without symptoms for about two months, was excised from

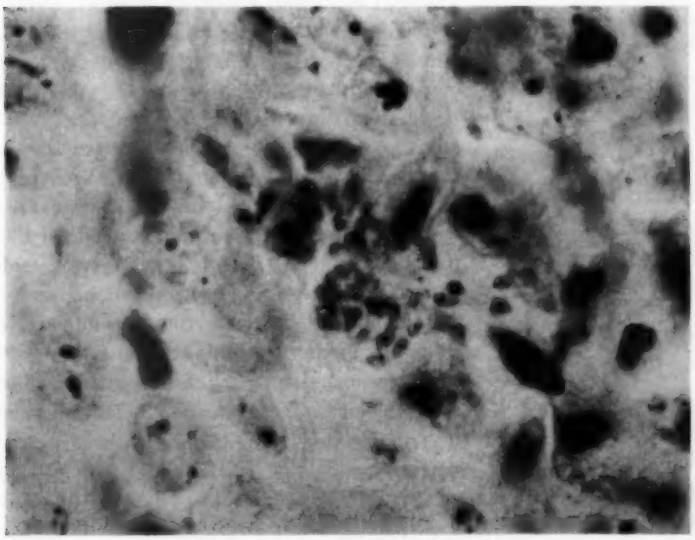


Fig. 13.—Mouse peripancreatic tissue: Toxoplasma in granuloma. Giemsa stain; reduced slightly from mag. × 1600.

the mother. This pink-tan node measured 1.2×0.9×0.5 cm. Microscopic examination showed only hilar and capsular fibrosis and reduction in size and number of germinal centers with vague follicular margins. A portion of this node was injected into two immature Swiss white mice, one of which died three days later of nonspecific pneumonia. The other mouse, with a negative peritoneal tap on the fourth day, was killed on the sixth day, and microscopic examination of both mice yielded negative results.

Sterile blood serum of the mother was repeatedly sent to the Communicable Disease Center of the U. S. Public Health Service, Chamblee, Ga. Titers against Toxoplasma, by the methylene-blue dye method of Sabin and Feldman,[16] were as listed in the accompanying Table.

Dye Titers of Mother, Delivered Feb. 24, 1953

Date	Titer
3/28/53	1:4,096
10/18/53	1:16,000 to 1:64,000
3/24/54	1:16,384
11/26/54	1:1,024

The mother, last interviewed Nov. 26, 1954, has remained entirely well, without recurrence of lymphadenopathy. She has not become pregnant again.

A sample of the mother's blood, drawn March 24, 1954, was examined by Dr. Roy T. Fisk, of Hyland Laboratories. He reported that her blood type was Group A, Rh-positive (CDe/cde) and that there were no irregular isoagglutinins or evidence of isosensitization.

COMMENT

In suspected cases of congenital or acquired toxoplasmosis, the mere finding of organisms consistent with Toxoplasma in stained tissue sections is not unequivocal proof of toxoplasmal infection.[5] Sabin [17] states that the cardinal characteristics of T. gondii are as follows: typical morphology and staining characteristics of intracellular and extracellular forms (in stained impression films); pathogenicity for mice, guinea pigs, rabbits, chicks, etc.; obligate intracellular parasitism; immunologic relationship with established strains. In our case, we believe that congenital toxoplasmosis of the infant is adequately proved by the morbid anatomy of the brain, chorioretinitis, and finding of intracellular and extracellular parasites. That the mother had an acute acquired toxoplasmal infection seems certain, on the basis of the sequential dye titers at significant levels. It is doubtful, though not excluded, that her infection of Skene's glands in 1951 was due to Toxoplasma. The finding of apparent organisms in the mother's lymph node, with the finding of apparent Toxoplasma in a granuloma of the injected mouse, is strong presumptive evidence that the mother's lymphadenitis was a manifestation of toxoplasmal infection. We did not attempt successive blind mouse passages, which in some cases may be necessary for diagnosis.‖ Feldman [6] has never encountered spontaneous toxoplasmosis in laboratory-reared mice.

In congenital toxoplasmosis, an erythroblastosis-like pathologic picture has been noted several times.¶ The mother's serum showed no evidence of isosensitization 13 months post partum, which finding tended to support Frenkel and Friedlander, who in their valuable monograph attributed the extramedullary hemopoiesis to the stimulus of infection.[5]

Siim [8] reported in 1952 the case of a 29-year-old man with a history of fever, chills, and jaundice with generalized lymphadenopathy. The peripheral blood showed lymphocytosis with large lymphocytes somewhat resembling those seen in infectious mononucleosis. Inguinal lymph node biopsy, about three months after onset of symptoms, disclosed a blurring of the follicular architecture, with hyperplasia of the reticulum cells of the follicular centers. The cells were large and pale with dark-staining nuclei showing many typical mitoses. Among the reticulum cells there were many basophilic granules of varying size and shape (Siim did not consider these to be parasites). Although Toxoplasma was not isolated from the node by three mouse

‖ References 5 and 6.

¶ References 5, 10, and 13.

passages, the patient's blood serum showed rising dye titers to 1:6520 and rising complement-fixation tests for Toxoplasma to 1:128. Siim later reported isolation of Toxoplasma by animal inoculation from the cervical lymph node of a 17-year-old girl with generalized lymphadenopathy.[15] Gard and Magnusson [12] reported the case of a 23-year-old woman who developed fever and generalized lymphadenopathy, predominantly cervical. Her toxoplasmin skin test was strongly positive, and her dye titer was 1:50. She became pregnant shortly after onset of infection but delivered a normal infant. Biopsy of a lymph node of the mother did not provide a definite morphologic diagnosis, but on review the sections showed, in macrophages, organisms that were possibly Toxoplasma. Animal inoculation was not mentioned. Pinkerton # recently found morphologically typical Toxoplasma in the lymph node of an American woman with a high titer of dye test antibody. In a clinicopathologic study of five cases of toxoplasmosis with literature review in 1946, Callahan, Russell, and Smith [13] stated that the lymph nodes contained parasites in only one instance, without significant pathologic change in the surrounding tissue. Armstrong and MacMurray [14] reported the case of a 44-year-old Negro man, a truck driver in Washington, D. C., who developed weakness, malaise, anorexia, nausea, high fever, chills, and sweating. The liver was palpable, but there was no lymphadenopathy or splenomegaly. Cold agglutinins were present in titer of up to 1:1024. A single soft tender axillary lymph node subsequently appeared, from which biopsy tissue was taken and injected into a Rhesus monkey and a guinea pig, with negative results. However, injection into mice yielded T. gondii that on subsequent passage were pathogenic for mice, guinea pigs, and rabbits. The authors did not describe the histologic changes in the node. The patient's dye titers were 1:32,000 and, later, 1:250.

Judging from the lymph node changes described by Siim, which are similar to our findings, we are inclined to the belief that the changes are nonspecific and do not even suggest the etiologic diagnosis. Given a lymph node suspected to harbor Toxoplasma, a diagnosis could be made only by presumptive identification of the parasites in sections or imprint smears, supported by serologic evidence, or by isolation of Toxoplasma by animal inoculation, including blind passages if necessary.

The handful of cases thus far reported in which Toxoplasma has been found in, or isolated from, human lymph nodes, support the view that congenital toxoplasmosis is transmitted from a mother with a recent generalized infection that is subclinical or clinically insignificant. Sabin has well stated [17] that it may be that toxoplasmosis causes a great deal of infection but very little disease.

The literature supports the conclusion that a mother who has been delivered of an infant with toxoplasmosis is in no practical danger of infecting a subsequent infant. Frenkel and Friedlander [5] stated in 1951 that no such instance had been reported. Sabin, Eichenwald, Feldman, and Jacobs [18] followed the records of 50 such women, who subsequently delivered one or more children that were not infected.

SUMMARY

The case is presented of a woman whose newborn infant died of congenital toxoplasmosis.

Antepartum course was characterized in the mother by asymptomatic cervical lymphadenopathy when six months' pregnant.

In a cervical lymph node biopsy specimen of the mother taken six and one-half weeks post partum, the microscopic changes were nonspecific and similar to those described in one other case by Siim. Two cells thought to contain intracellular Toxoplasma proliferative forms were found.

Organisms consistent with Toxoplasma were found in a white mouse injected with tissue from the node.

The mother's postpartum serum methylene-blue dye titers, against Toxoplasma,

Pinkerton, H., cited by Sabin.[17]

reached more than 1 : 16,000 and were typical of recent acute infection.

As for Toxoplasma involving human adult lymph nodes, the microscopic details have now been described twice, Toxoplasma has been presumptively found in stained microscopic sections four times, and the organisms have been isolated by animal injection from lymph nodes three times.

Available evidence indicates that congenital toxoplasmosis is transmitted by a mother who has had a recent generalized infection that is subclinical or clinically insignificant.

The erythroblastosis-like pathologic picture, reported by others and found in the infant of our case, was probably due to the stimulus of infection rather than to isosensitization.

Dr. J. K. Frenkel gave help, encouragement, and suggestions in this study. Mr. Lloyd Matlovsky, Medical Photographer, University of Southern California School of Medicine, prepared the photomicrographs.

REFERENCES

1. Nicolle, C., and Manceaux, L.: Sur une infection à corps de Leishman (ou organismes voisins) du gondi, Compt. rend. Acad. sc. **147**: 763-765, 1908.

2. Jacobs, L.: Biology of Toxoplasma, Am. J. Trop. Med. **2**:365-389, 1953.

3. Wolf, A., and Cowen, D.: Granulomatous Encephalomyelitis Due to an Encephalitozoon (Encephalitozoic Encephalomyelitis): New Protozoan Disease of Man, Bull. Neurol. Inst. New York **6**:306-371, 1937.

4. Wolf, A.; Cowen, D., and Paige, B. H.: Toxoplasmic Encephalomyelitis: 4. Experimental Transmission of the Infection to Animals from a Human Infant, J. Exper. Med. **71**:187-214, 1940.

5. Frenkel, J. K., and Friedlander, S.: Toxoplasmosis: Pathology of Neonatal Disease, Pathogenesis, Diagnosis, and Treatment, Public Health Service Publication 141, 1951.

6. Feldman, H. A.: Clinical Manifestations and Laboratory Diagnosis of Toxoplasmosis, Am. J. Trop. Med. **2**:420-428, 1953.

7. Siim, J. C.: Acquired Toxoplasmosis, J. A. M. A. **147**:1641-1645, 1952.

8. Siim, J. C.: Studies on Acquired Toxoplasmosis: 2. Report of a Case with Pathological Changes in a Lymph Node Removed at Biopsy, Acta path. et microbiol. scandinav. **30**:104-108, 1952.

9. Mellgren, J.; Alm, L., and Kjessler, A.: Isolation of Toxoplasma from the Human Placenta and Uterus, Acta path. et microbiol. scandinav. **30**: 59-67, 1952.

10. Beckett, R. S., and Flynn, F. J.: Toxoplasmosis: Report of 2 New Cases, with a Classification and with a Demonstration of the Organisms in the Human Placenta, New England J. Med. **249**: 345-350, 1953.

11. Neghme, A.; Thiermann, E.; Pino, F.; Christen, R., and Agosin, M.: Toxoplasmosis humana en Chile, Bol. parasitol. chil. **7**:6-8, 1952.

12. Gard, S., and Magnusson, J. H.: Glandular Form of Toxoplasmosis in Connection with Pregnancy, Acta med. scandinav. **141**:59-64, 1951.

13. Callahan, W. P., Jr.; Russell, W. O., and Smith, M. G.: Human Toxoplasmosis: Clinicopathological Study with Presentation of 5 Cases and Review of the Literature, Medicine **25**:343-397, 1946.

14. Armstrong, C., and MacMurray, F. G.: Toxoplasmosis Found by Recovery of Toxoplasma Gondii from Excised Axillary Gland, J. A. M. A. **151**:1103-1104, 1953.

15. Siim, J. C.: Histological Picture in Lymph Nodes in Acquired Toxoplasmosis, Schweiz. Ztschr. allg. Path. **16**:506-508, 1953.

16. Sabin, A. B., and Feldman, H. A.: Dyes as Microchemical Indicators of a New Immunity Phenomenon Affecting a Protozoon Parasite (Toxoplasma), Science **108**:660-663, 1948.

17. Sabin, A. B.: Toxoplasmosis: Current Status and Unsolved Problems, Am. J. Trop. Med. **2**: 360-364, 1953.

18. Sabin, A. B.; Eichenwald, H.; Feldman, H. A., and Jacobs, L.: Present Status of Clinical Manifestations of Toxoplasmosis in Man, J. A. M. A. **150**:1063-1069, 1952.

Lymphosarcoma of the Female Mammary Gland

SAUL KAY, M.D., Richmond, Va.

Lymphosarcoma primarily involving the breast is a pathologic curiosity. Undoubtedly a number of cases of lymphosarcoma of the breast do occur in which the involvement is part of a generalized disease process, but the exact incidence would be difficult to evaluate from the literature for obvious reasons. This report deals with a case of lymphosarcoma occurring simultaneously in both breasts and apparently arising primarily in both of these organs without the presence clinically of the disease in lymph nodes or other hematopoietic organs.

REVIEW OF THE LITERATURE

The first reference to this entity appears to be that of Elsberg [4] in 1914. The author reported a case of a woman with multiple tumors in both breasts, occurring two months after she started to nurse. No lymph nodes were affected. The lesions were locally excised, and no follow-up was available. McWilliams' case,[9] published in 1912, was actually a case of acute lymphatic leukemia, with leukemic nodules in both breasts and right axillary lymph nodes. Breucken and Schleiter [2] in 1926 reported a case of a 34-year-old white woman with lymphosarcoma involving both breasts. The patient died six months after onset of symptoms, and as autopsy showed lymphosarcoma of lymph nodes, uterus, ovaries, kidneys, and labia, as well as the breasts, it is possible that the primary disease was not in the breasts.

Received for publication Aug. 29, 1955.

From the Department of Surgical Pathology, Medical College of Virginia.

One year later, Thür [15] reported two unusual sarcomas of the breast. His first case seems to be a typical lymphosarcoma of the left breast in a 52-year-old white woman. There was no other tumor site anywhere else in the body, and the patient was without recurrence one and one-half years following simple excision and postoperative radium therapy. The author could find no similar case in a review of the literature.

Seidemann's case [14] in 1928 concerned a 48-year-old woman, but the patient had generalized disease and, by the author's own admission, the primary site was thought to be in the tonsil.

D'Annoy and Wright [3] discussed 11 cases of sarcoma of the breast, of which 1 was a lymphosarcoma. The patient was 39 years of age and was treated by a left radical mastectomy. No follow-up was available.

Schreiner and Thibaudeau [12] reported seven cases of sarcoma of the breast, one of which proved to be a lymphosarcoma. This occurred in the breast of a 69-year-old woman who was treated by radical mastectomy and x-ray therapy. Though the patient developed widespread metastases, she was controlled for almost five years, after which time she was lost to follow-up.

Fox [5] in 1934 presented four new cases of lymphosarcoma of the breast. Three of the cases were followed, and all three patients died within five months.

In Geschickter's [7] text, dealing only with conditions of the breast, the author recounts his personal experience with eight cases. The ages ranged from 15 to 63 years, and two of the youngest patients had bilateral disease. Only one of four cases responded to x-ray therapy. In six of the eight cases,

the course was rapidly fatal. Only one patient was alive two years after x-ray therapy. In the cases with bilateral involvement, the disease developed during, or shortly after, pregnancy, and both patients died within four months after the diagnosis was made.

Harrington and Miller [8] published their experience with this condition at the Mayo Clinic in 1940. They subdivided cases into three categories: (1) involvement of the mammary gland as part of a widespread primary process; (2) a neoplastic process apparently localized to the breast but with subsequent manifestations of systemic dissemination; (3) a truly localized lymphosarcoma of the breast. Two cases were presented in the second and third categories, respectively. The first case was that of a 28-year-old woman with nodules in both breasts. A right radical mastectomy showed multiple tumor foci without regional node involvement. The nodule in the left breast was only locally excised. Within a short time a mediastinal mass was discovered by a chest roentgenogram. Radiation therapy was given, but death occurred seven weeks after the patient was first seen. The second case was that of a 38-year-old woman with a 3 cm. nodule in the left breast. This was proved to be a lymphosarcoma, and treatment consisted of a radical mastectomy followed by radiation. The patient was living and well six years after surgery.

In 1944, Adair and Herrmann [1] proposed a classification of lymphosarcoma of the breast. The neoplasm was subdivided into primary and secondary types. The primary varieties were further subdivided into localized unilateral and diffuse bilateral disease. The localized unilateral form may have a single focus or multiple foci. The diffuse bilateral conditions were previously reported principally in the Italian literature. The disease manifests itself early in pregnancy with death shortly after parturition. Of the localized unilateral entities, the authors were able to collect 5 cases from among 10,964 breast conditions studied at Memorial Hospital of New York City within the past 20 years. The patients' average age was approximately 50 years. A single focus was found in four cases. Three cases were diagnosed as reticulum cell lymphosarcoma, one as follicular lymphosarcoma, and one as consistent with lymphosarcoma. On the basis of follow-up studies from three months to seven years, the authors concluded that radical mastectomy was the treatment of choice, provided the disease was localized and confined to one breast.

In 1948, Pohl [10] published a case of a lymphocytic tumor involving the breast of a 30-year-old woman. The breast was removed and showed lymphoid infiltration of the mature cellular type. The appearance was seemingly benign. Shortly after operation, and despite a course of radiotherapy, there was widespread local recurrence, with clinical evidence of metastases in the mediastinum and uterus. The use of additional radiotherapy was followed by a rapidly downhill course. Autopsy showed metastases in the myocardium and mediastinum, the cellular infiltrate being entirely similar to that noted in the breast. The pericardium, kidneys, liver, spleen, lymph nodes, bone marrow, and uterus had a lymphosarcoma-like appearance.

The most recent reference appears to be that of Fruhling and Le Gal.[6] The authors found 30 cases of sarcoma during a period when 2000 cases of carcinoma were diagnosed, an incidence of 1.5%. The sarcomas were subdivided in a rather complicated fashion into *sarcomes histiocytaires, leucocytaires, fibroblastiques, reticulaires, musculaires, lipoblastiques,* and *endothéliaux*. The *sarcomes leucocytaires* seem to represent cases of lymphosarcoma of the reticulum cell and lymphocytic cell types. There were six examples in this group, and three cases of the latter type (*sarcomes lymphoblastiques*) appear identical to the present case. Of these three cases, one was a man who died of the disease, another was lost to follow-up, and the third was well three years later.

The following case report exemplifies the rare phenomenon of diffuse bilateral disease according to Adair and Herrmann's [1] classification. The case illustrates well the onset of bilateral neoplasm in the breasts apparently

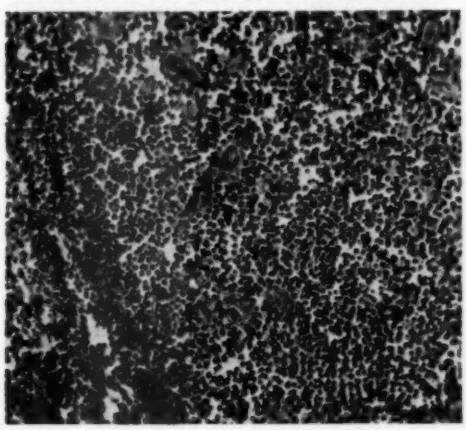
Fig. 1.—Clinical appearance of patient on admission.

during pregnancy, the failure of lactation following delivery, and the rapid ultimate fatal outcome.

REPORT OF A CASE

The patient, a 28-year-old Negro woman, was admitted to St. Philip Hospital Oct. 28, 1953, because of painful enlargement of both breasts, fever, and headache. She had just delivered her third child two weeks prior to admission and had nursed the infant while in the hospital during the postpartum period. The infant, however, was unable to get satisfactory feedings, and the patient stopped nursing the infant on discharge from the hospital. The patient had also complained of persistent headache without nausea or vomiting during the past three to four months, for which she sought relief by administering a preparation containing acetanilid and sodium bromide (Bromo-Seltzer).

Fig. 2.—Low-power view of biopsy tissue from left breast; reduced about ⅓ from mag. × 100.



The past history revealed no complications of her previous pregnancies or lactations. The patient had apparently enjoyed good health and denied any history of illness, injuries, or operation.

Physical examination revealed a well-developed, well-nourished Negro woman complaining of pain in both breasts. These were extremely engorged, warm, tense, and hardened throughout (Fig. 1). There were generalized tenderness and pitting edema to a moderate degree. No fluctuation was elicited. No milk could be expressed from either nipple, but a small amount of blood exuded from the right nipple on manual compression. The body temperature was 102 F.

The laboratory findings showed a hemoglobin of 10.6 gm. per 100 cc., 8100 white blood cells, and a normal differential count. The urinalysis was negative.

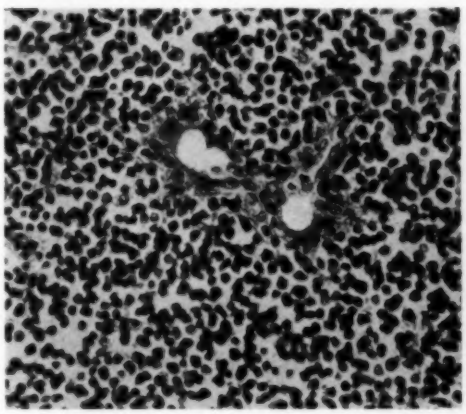


Fig. 3.—Higher magnification of lesion illustrated in Figure 2; reduced about ⅓ from mag. × 200.

The clinical impression was bilateral mastitis, and the patient was placed on supportive therapy with administration of crystalline procaine penicillin G (Crysticillin) and testosterone. Only slight reduction in size of the breasts and slight amelioration of symptoms resulted. On the third hospital day the patient developed severe occipitofrontal headache with bilateral papilledema and supraorbital tenderness. A spinal tap revealed clear fluid, a pressure of 520 mm. water, 50 cells per cubic millimeter with 98% lymphocytes, and 11 mg. of protein per 100 cc. The headache increased in intensity, and the patient developed vomiting and a positive Babinski sign on the right. A ventriculogram showed several small ventricular defects compatible with multiple cerebral lesions. On Nov. 12, a biopsy of the right breast was performed (see pathologic report below). On the same day the patient developed left hemiparesis and became stuporous. Four days later biopsy of the left breast

was performed. As the patient's condition was rapidly going downhill, an emergency craniotomy was performed for the purpose of decompression. On Nov. 18, biopsy specimens of the skull, dura, and brain were obtained. The patient died a few hours after the craniotomy. Permission for autopsy was not obtained.

PATHOLOGIC FINDINGS

Biopsy Specimen of the Right Breast.—This specimen consisted of three pieces of pink-tan tissue, the largest 5 by 4 by 3 mm. All were encased in fat. Section showed fragments of fat and fibrous tissue. In the fibrous stroma there were dense aggregates of lymphoid elements which permeated between prominent capillary blood vessels lined

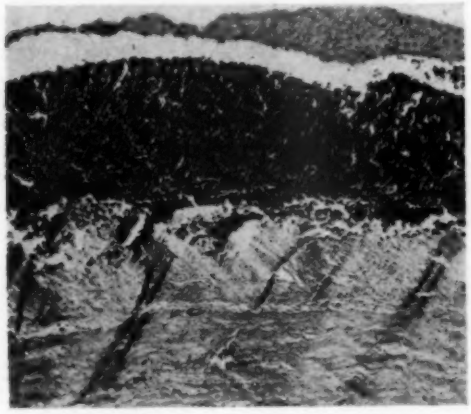


Fig. 4.—A plaque of metastatic tumor in the dura; reduced about ⅓ from mag. × 70.

by swollen endothelial cells. No actual mammary tissue was identified. These lymphoid cells consisted primarily of small and large lymphocytes and appeared to be neoplastic rather than inflammatory cells. Mitoses were occasionally noted. In addition, the lymphoid cells permeated between lobules of fat in a diffuse and haphazard fashion.

Biopsy Specimen of Left Breast.—The specimen consisted of an elliptical piece of skin and subcutis 5 by 1.5 cm. covering a wedge-shaped section of an ill-defined tumor mass attached to the subcutaneous tissue. The tumor was grayish-white and firm and measured approximately 5.5 by 3.3 by 2 cm. The overlying skin was freely movable. Section showed the tumor to be composed of diffuse, monotonous sheets of cells resembling lymphocytes (Fig. 2). A few mammary ducts could be identified within the lymphoid tumor (Fig. 3). A few isolated breast ductules could also be seen above the tumor mass in the subcutaneous tissue.

Tissue Secured During Intracranial Decompression.—The specimens were submitted as biopsy samples of bone, dura, and brain. The bone consisted of approximately eight irregular pieces of bone, the largest 5 mm. in maximal diameter. The dura consisted of two irregular pieces of flat tissue, the larger measuring 2 by 1 cm. There was some roughening of the surfaces. The brain tissues consisted of six fragments, the largest 4 mm. in maximal diameter. Sections of the bone and dura showed neoplastic elements similar to those already described in the breasts (Fig. 4). Mitoses were rather frequent. The brain was free of neoplasm.

Diagnosis.—Lymphosarcoma of female mammary gland, bilateral, with metastases to dura and skull (left temporal region).

COMMENT

The problem of verification of true primary lymphosarcomas of the breast is not easily resolved, since few cases are adequately documented to allow their acceptance as bonafide primary tumors. Unfortunately many of the cases referred to in this paper are not illustrated.* Some of the cases† represent merely diagnoses, and adequate details are lacking.

Ideally, a patient with a diagnosis of primary lymphosarcoma of the breast should have an easily discernible and histologically proved focus in the mammary gland without evidence of disease in the hematopoietic organs. Involvement of the hematopoietic system, as well as of the visceral and other organs, may occur at a later date but should be obviously secondary, as evidenced by the clinical facts and the course of the disease. Finally, careful follow-up of the patient is mandatory in so-called clinical "cures," and

* References 3, 4, 7, and 10.

† References 3, 5, and 12.

necropsy should be performed in those patients who died of the disease.

The inability to obtain permission for autopsy in the present case, therefore, leaves the issue of primary versus secondary tumor also unsettled. There are only the clinical facts for guidance, and these, I believe, are at least overwhelming. There was no clinical evidence of involvement of the lymph nodes or spleen, and peripheral blood studies ruled out the possibility of leukemia, though the aleukemic form could not definitely be excluded. There was obvious involvement of the central nervous system, and the dura may have been infiltrated with tumor at the time the patient complained of headaches three to four months prior to admission. The date of breast involvement by tumor would be difficult to ascertain because of the physiological enlargement expected with the patient's third pregnancy. It would hardly seem likely, however, that the dura harbored the primary growth and that the appearance of both breasts was due to bilateral metastases. The enormity of the mammary glands and the diffuseness of the lymphoid infiltrate throughout the mammary stroma available for histologic study would seem to rule out this possibility.

SUMMARY

A case of apparent primary lymphosarcoma of both female mammary glands is presented.

The literature dealing with previously reported and similar cases is reviewed.

Emphasis is placed on the difficulties arising in establishing the true primary nature of the disease.

Dr. Cushman D. Haagensen read the original manuscript and offered many useful suggestions.

REFERENCES

1. Adair, F. E., and Herrmann, J. B.: Primary Lymphosarcoma of the Breast, Surgery **16**:836-853, 1944.

2. Breucken, A. J., and Schleiter, H. G.: Lymphosarcoma Involving Both Breasts, Atlantic M. J. **29**:693-696, 1926.

3. D'Annoy, R., and Wright, R. W.: Sarcoma of the Breast, Ann. Surg. **92**:1059-1066, 1930.

4. Elsberg, C. A.: Multiple Lymphosarcoma of Both Breasts, Ann. Surg. **60**:767, 1914.

5. Fox, S. L.: Sarcoma of the Breast, Ann. Surg. **100**:401-421, 1934.

6. Fruhling, L., and Le Gal, Y.: Les Sarcomes vrais primitifs de la mamelle, Bull. Assoc. franç. étude cancer **38**:477-496, 1951.

7. Geschickter, C.: Diseases of the Breast, Ed. 2, Philadelphia, J. B. Lippincott Company, 1946, pp. 386-387.

8. Harrington, S. W., and Miller, J. M.: Lymphosarcoma of the Mammary Gland, Am. J. Surg. **48**:346-352, 1940.

9. McWilliams, C. A.: Bilateral Lymphosarcoma of the Breasts, Ann. Surg. **55**:439-441, 1912.

10. Pohl, W.: Bösartiger Verlauf einer zunächst gutartigen Geschwulst (Lymphocymatosa mammae), Wien. klin. Wchnschr. **60**:444, 1948.

11. Rogers, H., and Flo, S.: Sarcoma of the Breast, New England J. Med. **226**:841-844, 1942.

12. Schreiner, B. F., and Thibaudeau, A. A.: Sarcoma of the Breast: Report of 7 Cases, Ann. Surg. **95**:433-439, 1932.

13. Schultz-Brauns, O., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Springer-Verlag, 1933, Vol. 7, Pt. 2, pp. 232-233.

14. Seidemann, H.: Seltener Mammatumor, Monatsschr. Geburtsh. u. Gynäk. **78**:310-313, 1928.

15. Thür, W.: Zur Kenntnis seltener Geschwulstformen der weiblichen Brustdrüse (Lymphosarkom, Spindelzellensarkom), Arch. path. Anat. **265**:96-102, 1927.

Experimental Renal Infarction

I. Changes in Succinic Dehydrogenase and Cytochrome Oxidase Activity

THOMAS L. GAVAN, M.D.
and
NATHAN KAUFMAN, M.D., Cleveland

In the past extensive studies have been carried out on experimental infarction, and the resulting histologic changes have been observed carefully.* More recently attention has been drawn to the chemical and histochemical changes in the kidneys following partial or complete anoxia.† The present report deals with changes in the activity of two oxidative enzymes, succinic dehydrogenase and cytochrome oxidase, studied by histochemical methods following complete renal artery ligation. These two enzymes play a vital role in the complex tricarboxylic acid cycle of Krebs which serves as the terminal pathway in the oxidation of carbohydrates, fatty acids, and amino acids. Succinic dehydrogenase participates specifically in the oxidation of succinate to fumarate. In the absence of this enzyme the cycle is broken at this point. Cytochrome oxidase is necessary for the reoxidation of the cytochromes which are reduced during the oxidation of such components of the Krebs cycle as isocitrate, succinate, and malate. When there is extensive interference with the oxygen supply, as in vascular occlusion, these enzymes, among others, cease to function and cell death results.

Received for publication July 25, 1955.

From the Departments of Pathology, Cleveland City Hospital and Western Reserve University School of Medicine.

* References 1 through 4.

† References 5 and 6.

MATERIAL AND METHODS

Thirty-six albino male rats of the Sprague-Dawley strain, weighing approximately 225 gm., were used. Each animal was placed under light ether anesthesia and the perinephric fat dissected from the left kidney. The left renal artery then was exposed and ligated with a linen suture. If additional or aberrant arteries were found, they too were tied. Within 30 seconds after ligation the kidneys became swollen and took on a bluish hue. The opposite renal artery was left intact so that the right kidney could serve as a control. The abdomen was then closed with linen suture. The animals were killed in groups of four at intervals of 1, 2, 4, 8, 12, 18, 24, 36, and 48 hours after ligation.

Tissue from each kidney was immediately frozen at —70 C. Frozen sections were made at 75μ. When thinner sections were cut, the friable, infarcted tissue fragmented readily and satisfactory representative sections could not be obtained.

Histochemical studies for cytochrome oxidase activity were made. The sections were prepared according to the method described by Gomori,[7] employing the Nadi reaction. Sections were incubated in a mixture of 12.5 cc. of 0.5 M phosphate buffer, pH 7.4, 1.0 cc. of 1% α-naphthol in 40% ethyl alcohol, and 1.0 cc. of 1% dimethyl-*p*-phenylenediamine hydrochloride for 10 minutes at 37 C. The cytochrome oxidase was inactivated in duplicate sections using 1% sodium cyanide solution. This was done in order to make certain that the reaction in the original section was due to the cytochrome oxidase and not to oxidation of the Nadi reagent by other means.

Average Enzymatic Activity of Succinic Dehydrogenase and Cytochrome Oxidase Following Complete Renal Artery Ligation

Time	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	18 Hr.	24 Hr.	36 Hr.	48 Hr.
Average Activity									
Succinic dehydrogenase	++++	++++	+++	+++	++	++	+	0	0
Cytochrome oxidase	++++	++++	+++	+++	+++	+++	++	+	0

Succinic dehydrogenase activity was determined according to the method described by Seligman and Rutenberg,[8] modified by using 2, 3, 5-triphenyltetrazolium chloride as an oxidant. An insoluble red formazan dye is produced at the site of enzyme activity.

RESULTS

The activity of cytochrome oxidase and succinic dehydrogenase in the kidneys was graded from 0 to 4+ by comparison of the infarcted left kidney with the undisturbed right kidney which served as a control. In those graded 4+, the amount of activity in the experimental kidney was the same as in the control. Those graded zero showed complete absence of enzyme activity in the infarcted tissue. Not considered in the grading was a narrow, persistent zone of subcapsular, cortical enzymatic activity.

At one and two hours after renal artery ligation, no decrease in enzyme activity was observed, and the kidneys were graded 4+. However, after four hours, a definite decrease in activity was noted for both enzymes. The first changes were seen in the distal convoluted tubules. Throughout the remainder of the series a steady decrease in enzymatic activity occurred, the succinic dehydrogenase activity diminishing at a more rapid rate. Thus, at four and eight hours the infarcted kidneys were graded as 3+ for both enzymes. At 12 and 18 hours they were graded as 2+ for succinic dehydrogenase and 3+ for cytochrome oxidase. At 24 hours there was 1+ succinic dehydrogenase and 2+ cytochrome oxidase activity. At 36 hours no succinic dehydrogenase activity was detected, whereas the cytochrome oxidase still showed 1+ activity. However, at 48 hours there was no detectable cytochrome oxidase activity in the infarcted tissue.

COMMENT

In this experiment no changes in succinic dehydrogenase and cytochrome oxidase activity in the kidney were observed during the first two hours after renal artery ligation. At four hours definite changes for both enzymes were seen in the distal convoluted tubules. From then on, the decrease in activity of the two enzymes was not identical; the succinic dehydrogenase decreased more rapidly and was the first to disappear. A similar relationship was noted by Berenbom and co-workers [9] in their study of necrosis in the mouse liver. Using tissue homogenates, they found that succinic dehydrogenase activity decreased at a more rapid rate than that of cytochrome oxidase during the first six hours. This relative difference in the disappearance of enzymatic activity of the two enzyme systems is of interest, since cytochrome oxidase functions as a terminal oxidative enzyme serving many other enzyme systems. Its destruction, therefore, signals complete slowdown and standstill of the Krebs tricarboxylic cycle.

Peters and Farber [5] report that after occluding a branch of the renal artery in rats for a period of three hours there is complete cessation of succinic dehydrogenase activity in the infarcted area. In our experiments done under somewhat different conditions, complete disappearance of succinic dehydrogenase activity was not seen until 36 hours had elapsed. Cytochemical studies regarding cytochrome oxidase activity in renal infarcts are unavailable for comparison with our results.

After 48 hours a narrow subcapsular cortical zone of enzymatic activity still was present, although there was no activity elsewhere in the kidney, indicating that an adequate supply of blood reached this zone through the capsular vessels even though the perinephric fat had been removed previously. Goebel and Puchtler [6] noted a similar persistent zone in their study of the effect of renal artery ligation on the alkaline phosphatase and 5-nucleotidase activity.

SUMMARY

The left renal artery was tied in each of a group of 36 rats. There was a demonstrable decrease of succinic dehydrogenase and cytochrome oxidase activity at four hours. At 36 and 48 hours, respectively, there was no succinic dehydrogenase or cytochrome oxidase activity, except in a narrow subcapsular zone.

REFERENCES

1. Karsner, H. T., and Austin, J. H.: Studies in Infarction: I. Experimental Bland Infarction of the Kidney and Spleen, J. A. M. A. **57**:951-958 (Sept. 16) 1911.

2. Karsner, H. T., and Ash, J. E.: Studies in Infarction: II. Experimental Bland Infarction of the Lung, J. M. Res. **27**:205-224 (Nov.) 1912.

3. Karsner, H. T., and Ghoreyeb, A. A.: Studies in Infarction: III. Circulation in Experimental Pulmonary Embolism, J. Exper. Med. **18**:507-511 (Nov.) 1913.

4. Karsner, H. T., and Dwyer, J. E.: Studies in Infarction: IV. Experimental Bland Infarction of the Myocardium, Myocardial Regeneration and Cicatrization, J. M. Res. **34**:21-39 (March) 1916.

5. Peters, E. R., and Farber, E.: Histochemical Localization of Certain Oxidative Enzymes During the Production of Infarcts of the Rat Kidney, Am. J. Path. **31**:590-591 (May-June) 1955.

6. Goebel, A., and Puchtler, H.: Über das Verhalten der histochemisch nachweisbaren alkalischen Phosphatase und 5-Nucleotidase im Sauerstoffmangel und im Niereninfarkt, Arch. path. Anat. **326**:119-134 (Nov.) 1954.

7. Gomori, G.: Microscopic Histochemistry: Principles and Practice, Chicago, University of Chicago Press, 1952.

8. Seligman, A. M., and Rutenberg, A. M.: Histochemical Demonstration of Succinic Dehydrogenase, Science **113**:317-320 (March 23) 1951.

9. Berenbom, M.; Chang, P. I.; Betz, H. E., and Stowell, R. E.: Chemical and Enzymatic Changes Associated with Mouse Liver Necrosis in Vitro, Cancer Res. **15**:1-5 (Jan.) 1955.

News and Comment

GENERAL NEWS

Dr. Edwin F. Hirsch at Kansas City Conference.—Dr. Edwin F. Hirsch, of St. Luke's Hospital, Chicago, was visiting pathologist at the 33d Annual Fall Clinical Conference of the Kansas City Southwest Clinical Society, held in Kansas City, Mo., Oct. 3-6, 1955.

Trudeau Medal to Dr. William H. Feldman.—Dr. William H. Feldman, Professor of Pathology at the Mayo Foundation Graduate School of the University of Minnesota, has been awarded the Trudeau medal for meritorious contributions relating to the cause, prevention, and treatment of tuberculosis.

Robert A. Black Lecture.—The Third Robert A. Black Lecture of the La Rabida Jackson Park Sanitarium, Chicago, was given on Oct. 14, by Dr. Lewis Thomas, Professor of Pathology, New York University School of Medicine. Dr. Thomas discussed the origin and nature of fibrinoid.

Ludvig Hektoen Memorial Lecture.—The Second Ludvig Hektoen Memorial Lecture of the Hektoen Institute for Medical Research was given at the Cook County Hospital, Chicago, on Oct. 12, by Prof. Herwig Hamperl, Director of the Pathological Institute of the University of Bonn, Germany. He discussed the relationship of nutrition and disease.

A NEW SERIES IN

SEX EDUCATION

Titles in the new series

- PARENTS' PRIVILEGE
  for parents of young children of pre-school and early school age
- A STORY ABOUT YOU
  for children in grades 4, 5, and 6
- FINDING YOURSELF
  for boys and girls of approximately junior high school age
- LEARNING ABOUT LOVE
  for young people of both sexes (about 16 to 20 years of age)
- FACTS AREN'T ENOUGH
  for adults who have any responsibility for children or youth that may create a need for an understanding of sex education

PRICES

Note: Discounts apply to quantities of any single title, or to quantities of sets.

Quantity	Discount	Price
1	—	$ .50
10	10%	4.50
25	30%	8.75
50	40%	15.00
100	50%	25.00
1000	60%	200.00

Set of 5 titles, $2.25.

Distributed By
Order Department

AMERICAN MEDICAL ASSOCIATION
535 N. Dearborn St.
Chicago 10, Ill.

ORDER BLANK

Enclosed is $_____(no stamps) for the following pamphlet(s):

Title	Quantity
1. PARENTS' PRIVILEGE	_____
2. A STORY ABOUT YOU	_____
3. FINDING YOURSELF	_____
4. LEARNING ABOUT LOVE	_____
5. FACTS AREN'T ENOUGH	_____
Complete set of five	_____

Please send pamphlet(s) to:
(Please Print)

Name_____
Street_____
City_____
Zone_____ State_____

AP 11-55

Reconditioned Microscopes

All Standard Makes

B & L —— Spencer —— Leitz —— Zeiss

Spencer Vertical Binc. Research Circular Stage

Graduated Mechanical Stage Complete with Apochromats $425.00

Spencer 820 Microtome, $350.00

B & L Clinical Freezing Microtome (like new) $225.00

Pre-Med Types from $175.00

Various Accessories—What are your needs?

GUARANTEED REPAIRS

Free estimates 48 Hr. Service

Monroe Microscope Service

P. O. BOX 656

ROCHESTER 2 NEW YORK

Of interest to you and your patients

THE MENACE OF ALLERGIES

HOUSE DUST ALLERGY
by Karl D. Figley
8 pages, 15 cents

ASTHMA, A PROBLEM IN PREVENTION
by Alene S. and Robert P. Little
12 pages, 15 cents

WHAT WE KNOW ABOUT ALLERGY
by Louis Tuft
12 pages, 15 cents

PROTECTING YOUR CHILD FROM ALLERGY
by William Gayle Roberts
8 pages, 15 cents

AMERICAN MEDICAL ASSOCIATION
535 NORTH DEARBORN • CHICAGO 10

A.M.A. ARCHIVES OF INTERNAL MEDICINE

integrate your knowledge—subscribe to . . .

A. M. A. AMERICAN JOURNAL OF DISEASES OF CHILDREN

A. M. A. Archives of OTOLARYNGOLOGY

A. M. A. Archives of OPHTHALMOLOGY

A. M. A. Archives of PATHOLOGY

For a more complete reference shelf, we recommend these related journals. The variety of fields covered are especially appealing to the general practitioner with special interests. Each issue is important to your practice. Begin your subscriptions TODAY.

Department (I. M.)

AMERICAN MEDICAL ASSOCIATION
535 N. Dearborn St.
Chicago 10, Ill.

Begin my subscription(s) NOW, for:

A. M. A. .....

NAME .....

STREET .....

CITY ..... STATE .....

PARAGON STAINS **PARAMOUNT QUALITY**

PARAGON STAINING SOLUTIONS

For Tissue Sections

Dependable—Today; Tomorrow; Every Day

With Paragon Staining Solutions you obtain superbly stained tissue sections. The brilliance and sharpness of the stain without diffusion or unpredictable characteristics greatly facilitates diagnosis.

HEMATOXYLIN STAIN—PARAGON (aqueous alum hematoxylin). Made from our own formula. Yields vivid, sharply stained blue nuclei that are really blue—not off color or muddy. Extremely sharp staining and selective with no diffusion. Full bodied and strong. For a given staining time, repeatedly duplicates depth of staining from slide to slide—every day.

PS1101	Bottle (500 cc)	$2.25

EOSIN STAIN—PARAGON (alcoholic). A special eosin compound of our own preparation. Produces deep brilliant red counterstains. Packed in two forms—ready to use and concentrated (requiring the addition of 3 parts of 95% alcohol).

PS1201D	Bottle (500 cc) ready to use	$2.25
PS1201	Bottle (250 cc) for 1000 cc	3.00

ELASTIC FIBER STAIN—PARAGON. Our own resorcin-fuchsin modification of Weigert's Elastic Fiber Stain. Relieves the laboratory of the laborious work involved in the preparation of this important stain. Stains sharply with no diffusion into other tissue components.

PS1225	Bottle (250 cc)	$2.65

VAN GIESON STAIN—PARAGON. Especially designed to produce brilliant differential counterstaining with less tendency to wash out in rinsing alcohols.

PS1250	Bottle (250 cc)	$1.50

PARAGON MULTIPLE STAIN FOR FROZEN SECTIONS. Invaluable to the Pathologist where seconds count and the Surgeon waits for the diagnosis. A single solution which stains instantaneously yielding a hematoxylin-eosin like picture. No special technic. With Paragon Mounting Medium For Frozen Sections (water soluble) section is stained, mounted and under microscope in less than one minute.

PS1301	Paragon Multiple Stain For Frozen Sections	Bottle (50 cc)	$2.00
P451	Paragon Mounting Medium For Frozen Sections	Bottle (25 cc)	.50

Request samples on your institution letterhead.
Write for fully descriptive catalog number 1049 A which includes a descriptive section on staining technics.

All prices F. O. B. New York, New York, subject to change without notice.

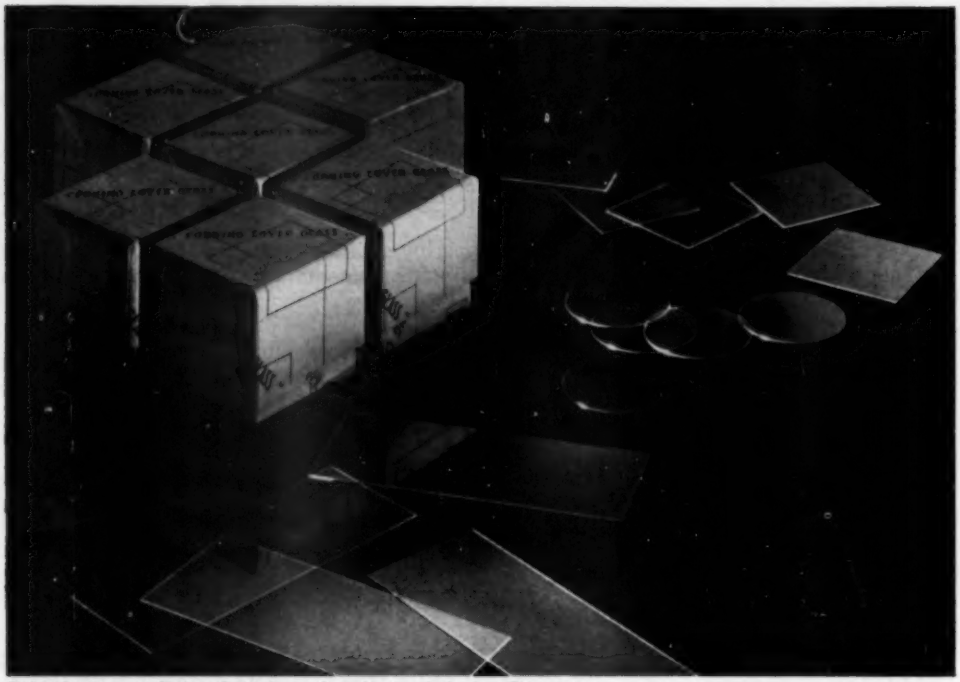
Manufactured exclusively by

PARAGON C. & C. CO., Inc. • 2540 Belmont Ave., New York 58, N. Y.

Cable Address: Wijeno, New York.

Write for details on the following Paragon Staining Solutions:

ACID FAST BACTERIA STAIN • CRYSTAL VIOLET STAIN • GRAM'S IODINE SOLUTION
SAFRANIN STAIN • LOEFFLER'S ALKALINE METHYLENE BLUE • ZIEHL-NEELSEN STAIN
WRIGHT'S STAIN • BUFFER SOLUTION FOR WRIGHT'S STAIN



Reduced tolerances eliminate borderline and overlapping thicknesses. For example, Corning No. 1 ranges from .13 to .16 mm. and No. 2 from .19 to .25 mm.

Corning brand Optical Micro Cover Glasses—

Now in 7 thinnesses – all uniformly flat

You can get Corning Cover Glasses in seven thinnesses—from 00 to 14-5.

And you can get any thinness in round, square, or rectangular shape.

The thinness and shape you choose will be as uniformly flat as only rigidly controlled machine production can make them. Our wide selection of critically defined thinnesses helps you choose the one best for the work you do. Flatness, of course, helps you prevent errors, lets you study the entire microscopic field with uniform ease.

To carry your ease of study even further, we make our cover glasses of *optical* grade glass. It's water white (you'll find no color tinge present) and crystal clear. There are no seeds, striae, bubbles, blisters or stones to block or distort your vision.

You'll find they last long, too. You can clean them repeatedly, subject them to any number of reagents; they'll come up shining.

For more attractive prices combine your Corning Cover Glass orders with your regular PYREX labware. Ask your regular laboratory supply house or write direct for complete information.

Corning Glass Works
Corning, New York

Corning means research in Glass

PYREX® laboratory ware

. . . the tested tool of modern research

IN THE DETECTION AND MANAGEMENT OF INFLAMMATORY CONDITIONS . . . C·R·P·A

Tests for C-reactive protein depend on a single factor . . . the presence of inflammation.

C·R·P·A

More Accurate than Sedimentation Rate Determinations[1]

"False positives" do not occur in tests for C-reactive protein because this abnormal protein appears only in patients with inflammatory conditions but is never present in normal serum.[2] Disappearance of CRP or changes in its concentration parallel more closely and more promptly variations in the patient's condition than usually evidenced by fluctuations in sedimentation rate.

●

C·R·P·A

More Easily Interpreted than Sedimentation Rate Determinations[3]

There is no "normal," therefore no equivocal zone of values in the interpretation of tests for C-reactive protein.[4] CRP tests are not invalidated in patients with congestion of the liver, in heart failure, anemia, cyesis, nephrotic syndrome, or changes in fibrinogen, serum albumen or globulin, when sedimentation rates may be misleading.

●

C·R·P·A

More Convenient to Perform than Sedimentation Rate Determinations[5]

The test for C-reactive protein may be performed at any time after obtaining a sample of the patient's serum. Since a large volume of serum is not necessary, blood may be drawn from a fingertip rather than from a vein. The simple technique employed in CRP determinations facilitates *routine* use in office and hospital.

C·R·P·A®

C-reactive Protein Antiserum (Schieffelin)

For complete descriptive brochure on techniques and materials required, send request to:

Schieffelin & Co. New York 3, N. Y. • Pharmaceutical and Research Laboratories since 1794

1. Shackman, N. H.: Heffer, E. T., and Kroop, I. G.: Am. Heart J. 48:509 (Oct.) 1954. • 2. Stollerman, G. H., and others: Am. J. Med. 15:645 (Nov.) 1953. • 3. McEwen, C.: M. Clin. North America 39:353 (March) 1955. • 4. Wood, H. F., and McCarty, M.: Am. J. Med. 17:768 (Dec.) 1954. • 5. McEwen, C., and Ziff, M.: M. Clin. North America (May) 1955, to be published.

fine "family" to get acquainted with...

technicon FIXATIVE
penetrates rapidly: completely stable: long-lived: precipitate-free
technicon DEHYDRANT
non-hardening: non-distorting: non-hygroscopic: non-volatile: lasts longer
technicon CLEARANT
non-hardening: non-volatile: non-hygroscopic: clears fat: lasts longer
technicon STAINS
highly selective: standardized characteristics: chemically balanced: never needs filtering
technicon MOUNTING MEDIUM
dries quickly: chemically inert: won't crystallize or darken: higher refractive index
technicon PARAWAY
paraffin solvent: won't "dry out": shrink or distort tissues: lasts longer

These are the members of the Technicon family of standardized histologic reagents. Many pathologists consider them the closest approach yet made to ideal media for routine laboratory use.

Not least among their outstanding characteristics is their common high flash point which makes them mandatory for use with Autotechnicon for Underwriter's Laboratory approval.

For details and specimen methods, let us send you our Bulletin R-2 "Standardized Histologic Reagents."

technicon®
standardized histologic reagents

TECHNICON CHEMICAL COMPANY, INC.
Chauncey, New York